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## Paddy straw compost application to increase nitrogen fixation on soybean cultivars

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# Paddy straw compost application to increase nitrogen fixation on soybean cultivars

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**Abstract.** Soybean is leguminous crop requiring large quantity of N nutrient. The N requirement in soybean crops can be fulfilled through N<sub>2</sub> fixation by root nodules. Root nodules can develop through the symbiosis of soybean crops with Rhizobium bacteria. This study aimed to identify the effects of paddy straw compost application on nitrogen fixation and physiological character in Baluran, Tanggamus, Seulawah, Grobogan cultivars of soybean crops. The study was conducted at regosol soil in Banguntapan, Yogyakarta. Result has showed that paddy straw compost dosage may increase soybean capability to perform nitrogen fixation, indicated by the variables of total root nodules, root nodule dry weight, and nitrogenase activity. Paddy straw application has not provided significant effect on total chlorophyll content, leaf greenness, water content in leaf, transpiration rate and CO<sub>2</sub> content in leaf, but has given significant effect on net assimilation rate of the crops.

## 1. Introduction

Soil serves as media for crop growth. Nutrient content in soil will affect crop growth. The advantages of soil organic matter (SOM) have been long known for crop growth and soil fertility. Since long time ago, farmers have utilized organic matter residue to increase soil fertility. These organic matters return into soil, both through natural and human-engineered systems as well. The application of organic matters has been proved to develop soil fertility and soil ecosystem health; and hence, it prevents possible environment pollution. In LEISA (low external input sustainable agriculture) system, external input usage is limited by optimizing the exploitation of available local resources; and the exploitation of external inputs is only needed to complement nutrient shortage in the ecosystem. The basic principle of LEISA are (1) to secure soil condition that supports crop growth, particularly through organic matter management and increasing soil microorganism; (2) to optimize nutrient availability and balance of nutrient flow, particularly through nitrogen fixation, recycle and external fertilizer application as complement; (3) to minimize loss due to solar radiation, air and water through microclimate management, water management and erosion control; (4) to minimize pest and disease invasion; (5) to perform mutual completion and synergy in exploiting genetic resources including the combination of integrated farming with higher level of functional diversity [1].

The addition of organic matters into soil, either through compost application, crop residue returning into soil, and soil cover crops may improve total stock of soil organic matters. Continuous farming practice will reduce total-C and total-N stocks; however, organic fertilizer application may maintain the balance of total-C and total-N stocks. Among the nutrients which crop requires, N turns out to be the most one that crops require. But, since the availability is lower due to its higher mobility, N nutrient is



easily lost through leaching and volatilization. Nitrogen fixation is important to supply N demand in sustainable farming system. The advantageous microbial availability in rhizospheres will increase soil fertility. Mycorrhiza is an example of microorganisms increasing water and nutrient absorbability in some crops. It also occurs with rhizobium, azotobacter, azospirillum which may serve as organic fertilizer and alternatives to chemical fertilizer. The symbiosis of soybean–mycorrhiza and soybean–rhizobium may improve crop growth, nodulation and biomass accumulation; therefore, it provides farmers with advantages due its reduced price of N and P fertilizers [2].

Nitrogen serves as factor limiting crop growth, particularly in soybean crop requiring a great deal of N for its growth. To supply this N demand, soybean has symbiotic relationship with bacteria developing root nodules, so that N<sub>2</sub> fixation may occur. Nitrogen fixation supplies more or less a quarter till a half of total nitrogen that legumes need [3]. In general, soybeans are cultivated following paddy harvesting time based on paddy-paddy-*palawija* or paddy-*palawija* soybean cropping patterns. Soybean that is directly cultivated after paddy may get nutrient residual advantages from paddy fertilization. In addition, paddy straws may be utilized as mulches or applied as compost and exploited as an organically fertilizing source. Paddy straws have not been optimally exploited as organic mass source in farming. Most of paddy straws are frequently burnt or exploited as livestock feed, material in paper industry or in mushroom farming, to enable soil tillage in the next cropping. Returning paddy straws into soil may restore some of nutrient to be transported during harvesting time. The straws to be used as the organic matter sources will be better if they are composted first to prevent nutrient competition between the composting microorganism and the crops.

Organic matters influence crop growth through its effects on nutrient availability for crop growth. In addition to serve as N, P, S nutrient sources through soil microorganism-created mineralization process, organic matters also function as energy source for N-fixing bacteria from the air [4]. Humate acid is known to increase legume's root nodulation that is inoculated with *Rhizobium trifolii*, *R. meliloti*, *R. leguminosarum*, and *R. japonicum* [5]. Rhizobium inoculation in soybean crop and manure application at coastal land may improve the growth and yield of soybean crops [6]. The paddy straw compost exploited in soybean cropping inoculated with *R. japonicum* is expected to be able in increasing root nodule development so that it also may improve the capability of soybean in nitrogen fixation. Compost yield (*rendement*) made from paddy straws shows more or less 60% of initial weight of the straws. The straw compost exploiting organic microbial decomposers contains the following nutrients: organic-C: 22.06 %; organic-N: 1.51 %; N-NH<sub>4</sub>: 0.05 %; N-NO<sub>3</sub>: 0.08 %; total-N: 1.64 %; P<sub>2</sub>O<sub>5</sub>: 0.53 %; K<sub>2</sub>O: 2.23 %; water content: 10.14% and C/N: 15 % [7].

## 2. Materials and Methods

The study was carried out in Banguntapan area, Bantul, Yogyakarta Special Province containing regosol soil. Materials involved composted-paddy straws, soybean legin (legume inoculant), Baluran, Tanggamus, Seulawah, Grobogan cultivars soybean seeds, and chemical substances utilized in plant tissue analysis. Instruments adopted were digital scale, ruler, thermohygrometer, lux meter, area meter, spectrophotometer, thermometer, microscope, oven, caliper, SPAD, chromatography and Photosynthetic analyzer LI-COR 6400.

The study consisted of two factors in Randomized Complete Block Design (RCBD) with three blocks. The first factor involved soybean cultivars (Baluran, Tanggamus, Seulawah, Grobogan). The second factor was paddy straw compost, consisting of four treatments, i.e. without paddy straw compost, with paddy straw composts of 15 tons·ha<sup>-1</sup>, 20 tons·ha<sup>-1</sup> and 25 tons·ha<sup>-1</sup>. Before planted, the soybean seeds were inoculated with soybean legin. The activity of nitrogen fixation by root nodules was observed according to the reduction of acetylene to ethylene by nitrogenase enzyme. The observation result was analyzed by using Analysis of Variance (ANOVA) at 5% significance level. To identify the significance of the treatments, the Duncan's Multiple Range Test (DMRT) was adopted at 5% significance level.

### 3. Results and Discussion

#### 3.1. Analysis on $N_2$ Fixation Capability

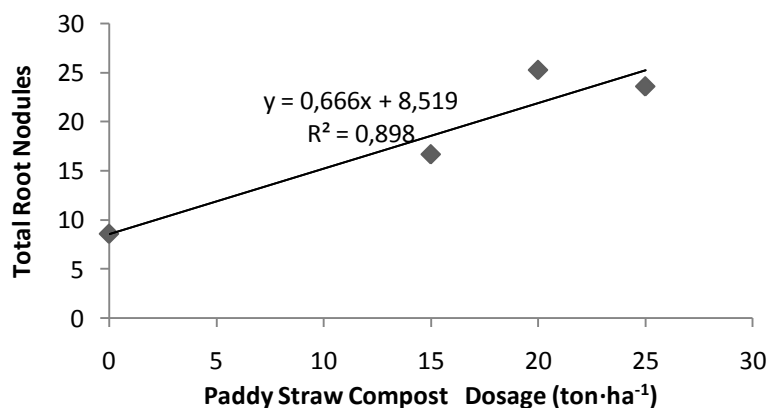
Identifying soybean cultivar infixing  $N_2$  can be seen from variable total nodules, nodule dry weight, nitrogenase activity, shoot N absorption. Result of variance analysis on total root nodules showed that no interaction was found between soybean cultivar and paddy straw compost dosage; however, paddy straw compost provided significant effect to increase total root nodules of soy bean crop.

**Table 1.** The root nodules and dry weight of root nodules (g) of soybean cultivar due to the effects of various paddy straw compost dosages.

Cultivar	Total root nodules	Dry weight of root nodules
Baluran	20,25 a	0,24 a
Tanggamus	23,50 a	0,24 a
Seulawah	14,75 b	0,13 b
Grobogan	15,58 b	0,18 ab
Paddy straw compost dosages (ton/ha)		
0	8,58 r	0,11 r
15	16,67 q	0,18 q
20	25,25 p	0,28 p
25	23,58 p	0,23 pq

Note: MAP= The values ended by similar letter in similar column shows insignificant result in Duncan's Multiple Range Test (DMRT) at significance level of 5%.

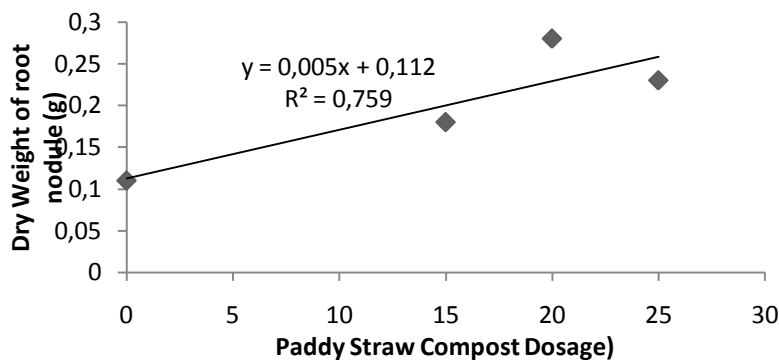
The increase of paddy straw compost dosage may also increase the average of total root nodules following regression equation  $y = 0.666x + 8.519$  ( $R^2 = 0.898$ ) as seen on figure 1.



**Figure 1.** The average of total root nodules in various paddy straw compost dosages.

The increase of paddy straw compost dosage also significantly may linearly increase the dry weight of soybean root nodules and is indicated by regression equation  $y = 0.005x + 0.112$  ( $R^2 = 0.759$ ). Result

of correlation analysis showed that there was positive and highly significant correlation between the dry weight of soybean root nodule and total root nodules ( $r = 0.80^{**}$ ). Total root nodules and the dry weight of soybean root nodule serve as indicators of nitrogen fixation capability. Soybean cultivar with greater number of root nodules and higher dry weight of soybean root nodule has higher nitrogen fixation capability compared to that with fewer root nodules.



**Figure 2.** The average of the dry weight of soybean root nodule (g) in various paddy straw compost dosages.

The activity of nitrogenase enzyme indicates the capability of soybean cultivar in fixing nitrogen. The nitrogen fixation occurs in bacteroid-containing root nodules. The synthesis of nitrogenase enzyme is done by bacteroid; this enzyme serves as catalyst reducing nitrogen to ammonium. The greater nitrogenase enzyme activity is, the greater soybean cultivar's capability in fixing nitrogen. Nitrogenase enzyme activity may be identified from the reduction activity of acetylene to ethylene in root nodules. The reduction of acetylene to ethylene is catalyzed by nitrogenase. The formed ethylene shows the activity of nitrogenase enzyme [8].

**Table 2.** The nitrogenase enzyme activity (mmol/crop/hour) of soybean cultivar due to the effects of paddy straw compost dosages.

Paddy Straw Compost Dosage	Cultivar				Average
	Baluran	Tanggamus	Seulawah	Grobogan	
0	0.017 c	0.018 c	0.025 c	0.024 c	0.021
15	0.021 c	0.025 c	0.019 c	0.028 c	0.023
20	0.119 a	0.058 bc	0.039 bc	0.017 c	0.058
25	0.077 b	0.032 c	0.024 c	0.023 c	0.039
Average	0.059	0.033	0.027	0.023	(+)

Note: The values ended by similar letter shows insignificant result in Duncan's Multiple Range Test (DMRT) at significance level of 5 %. (+): interaction is available.

Based on nitrogenase enzyme activity per crop (table 2) it is seen that organic matter, i.e. paddy straw compost may increase nitrogenase enzyme activity. Baluran cultivar applied with paddy straw compost of 20 ton·ha<sup>-1</sup> has showed highest and significant nitrogenase enzyme activity compared to Baluran, Tanggamus, Seulawah and Grobogan cultivars in various paddy straw compost dosage

applications. Compost application on soybean crop may increase acetylene reduction activity (ARA) as compost capability in improving root nodule formation. Compost application provides direct effect on symbiotic fixation through root nodule formation [9].

The capability of nitrogen fixation, among others, is indicated by shoot N content. The weight of shoot N content is closely related to crop capability in fixing nitrogen and root capability in nutrient absorption, among other, N nutrient. Crops with better root nodules and root system also have better nitrogen fixation capability and good nutrient absorption; hence, shoot N content is also higher. In this study, although total root nodules, root nodule dry weight, and nitrogenase activity increase due to paddy straw compost application, the increase is not followed by crop capability in absorbing N. Paddy straw compost dosage does not provide significant effect on crop's shoot N content. Crop's shoot N content is more determined by soybean cultivar characteristics. Cultivars with positive response to *Rhizobium* inoculation (Baluran and Tanggamus cultivars) show higher shoot N content [10].

**Table 3.** The shoot N weight (g/crop) of soybean cultivar due to the effects of various paddy straw compost dosages.

Paddy Straw Compost Dosage	Cultivar				Average
	Baluran	Tanggamus	Seulawah	Grobogan	
0	0.38	0.49	0.44	0.41	0.43 p
15	0.55	0.48	0.39	0.34	0.44 p
20	0.57	0.58	0.32	0.46	0.48 p
25	0.51	0.47	0.31	0.46	0.44 p
Average	0.50 a	0.50 a	0.37 b	0.42 b	(-)

Note: The values ended by similar letter shows insignificant result in Duncan's Multiple Range Test (DMRT) at significance level of 5 %. (-): no interaction available.

The application of paddy straw compost increases soil porosity, and so that it produces better the balance of air and soil humidity. Such a balance enables fixed-N and all nutrients from organic matter decomposition are more available as microorganism requires humidity and O<sub>2</sub> for maximum efficiency. Organic matters serve as energy sources to make N<sub>2</sub> symbiotic and non-symbiotic fixation previously unavailable to be available for crops.

### 3.2. Physiological character analysis

The applications of paddy straw compost on four soybean cultivars do not provide effects on the physiological character of soybean crop. Observations were conducted on chlorophyll content, leaf greenness level, CO<sub>2</sub> content in leaf, transpiration rate and photosynthesis rate.

**Table 4.** Total chlorophyll content (leaf mg·g<sup>-1</sup>), leaf greenness level, water content in leaf (mmol H<sub>2</sub>O·mol<sup>-1</sup>), transpiration rate (mmol H<sub>2</sub>O dm<sup>-2</sup>·s<sup>-1</sup>), CO<sub>2</sub> content in leaf (μmol CO<sub>2</sub>·mol<sup>-1</sup>), net assimilation rate (leaf area g·dm<sup>-2</sup>·week<sup>-1</sup>).

Compost Dosage (ton·ha <sup>-1</sup> )	I	II	III	IV	V	VI
	0	0.97 p	42.09 p	37.40 p	7591 p	277.6 p
15	0.93 p	42.97 p	34.98 p	9958 p	433.4 p	0.59 qr
20	0.98 p	43.24 p	37.29 p	8785 p	286.1 p	0.87 p
25	0.91 p	42.99 p	36.45 p	6565 p	278.7 p	0.80 pq

Note: The values ended by similar letter in similar column shows insignificant result in Duncan's Multiple Range Test (DMRT) at significance level of 5%.

- I: total chlorophyll content (leaf  $\text{mg}\cdot\text{g}^{-1}$ )
- II: leaf greenness level
- III: water content in leaf ( $\text{mmol H}_2\text{O}\cdot\text{mol}^{-1}$ )
- IV: transpiration rate ( $\text{mmol H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{s}^{-1}$ )
- V:  $\text{CO}_2$  content in leaf ( $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$ )
- VI: net assimilation rate (leaf area  $\text{g}\cdot\text{dm}^{-2}\cdot\text{week}^{-1}$ )

The application of paddy straw compost may increase crop's net assimilation rate. Result of correlation analysis indicated positive and significant correlation between net assimilation rate and total root nodules ( $r=0.35^*$ ), the dry weight of soybean root nodule ( $r=0.32^*$ ) and nitrogenase enzyme activity ( $r=0.28^*$ ). This shows that the increasing capability of nitrogen fixation will be followed by increasing net assimilation rate. Crops with higher capability in nitrogen fixation indicate better net assimilation rate.

#### 4. Conclusion

The application of paddy straw compost in *Rhizobium*-inoculated soybeans may increase soybean crops' fixing nitrogen. This is seen from increasing total root nodules, the dry weight of soybean root nodule and nitrogenase activity due to the application of paddy straw compost. The application of paddy straw compost does not provide significant effects on total chlorophyll content, leaf greenness level, water content in leaf, transpiration rate and  $\text{CO}_2$  content in leaf, but has given significant effect on net assimilation rate of the crop.

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Best regards,  
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on behalf of the Orgatrop Committee Chair Stefaan De Neve-Benito Heru Purwanto

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Thank you for your revision file.  
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Best regards,  
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Dear Ms Okti Purwaningsih

We have checked the revision of manuscript from you, but there are still something that need to fixed:

1. There is some references not listed in bibliography

So that the revision process of this manuscript can be completed quickly, please the above things can be followed in 1 x 24 hour.

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