Leaf anatomy response of several varieties of rice (*Oryza sativa* L.) to the application of silica fertilizers

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**Abstract.** Rice (*Oryza sativa* L.) requires large amounts of silica for its growth. This study aims to determine the response of leaf anatomy and agronomic performance of several rice varieties to the application of Silica fertilizer. The study was conducted in the first rainy season of 2018 (November 2017 to March 2018 in Semanu, Gunungkidul, Indonesia. The study was arranged in factorial, the first factor was 4 rice varieties (V1 = Inpari blast, V2 = Inpari 42, V3 = Ciherang, and V4 = Inpari 33), the second factor is the treatment of Silica fertilizer dosage (P1 = 250 kg / ha of Silica fertilizer; P2 = 500 kg / ha of Silica fertilizer, P3 = 1,000kg / ha of husk ash, and P4 = 750 kg / ha of Silica fertilizer). Observing agronomic traits including plant height, number of productive tillers, number of filled grains per panicle, and productivity). In addition to observing agronomic traits, microscopic observations of leaf anatomy were also carried out with thick upper and lower epidermal cells, cuticles in the upper and lower epidermis, mesophyll thickness in major and minor intervenous regions. The data obtained were analyzed for variance (ANOVA = Analysis of variance) and Duncan’s advanced test using SAS version 9.2. The results showed that the administration of silica fertilizer increased rice growth and productivity as well as increasing the thickness of the epidermis and upper and lower cuticles both in major and minor intervenous. The highest productivity was achieved by Inpari 42 varieties in the application of 750 kg Silica fertilizer/ha (9.69 ton/ha Harvested Dry Grain).

1. Introduction
Rice (*Oryza sativa* L.) was the staple food of most Indonesian people, the need for rice always increases with increasing population. Various efforts continue to be made by the government in overcoming the problem of rice needs. Efforts to develop paddy rice extensification are currently experiencing many obstacles, especially by the conversion of lowland to non-agricultural land. Intensification efforts to increase national rice production continue to be pursued by the government.

The low productivity of rice was caused by constraints due to environmental stress both biotic and abiotic. Therefore real efforts and actions are needed to overcome these obstacles. One effort that can be done was through improving agricultural cultivation techniques, namely by doing balanced fertilization. This is important because the productivity of rice plants is largely determined by the intake of nutrients obtained by plants from fertilizers.

The nutrient element that is rarely given or added to the soil in agricultural cultivation activities, especially rice plants is the micro-silica (Si) element. The need for rice plants will depend on the availability of Silica elements in nature.
Si as a non-essential nutrient is often not a concern. This was evident that so far there has been no addition of Si in the practice of rice farming [1]. Meanwhile, many agricultural lands in Indonesia experience leaching of micronutrients including Si, the availability of Si in the soil especially in the tropics was very low [2]. On the other hand, each time the rice crop transports Si between 100 - 300 kg/ha [3], rice plants can absorb silica in the range of 230-470 kg/ha [4]. Displacement of Si out of rice fields through the process of harvesting and washing without being accompanied by the addition of Si was a major factor causing the process of decreasing the content of Si available in the soil[3].

The low availability of Si in paddy soils was one of the causes of decreased productivity of rice plants [5]. This was in line with opinion[6], the decrease in Si available in the soil for plants was likely to be closely related to the decline in rice crop productivity.

Silica is one of the functional elements that have an important role in increasing plant growth, especially the Graminae family group such as rice [7]. Silica was absorbed by plants in the form of mono silicic acid into the plant through xylem and transported together with water, was transplanted to the leaves then the water evaporates and silica is stored in the epidermal layer of leaves in the form of amorphous silica [8]. Si’s role in increasing the growth and production of rice was caused by the improved photosynthesis system because the leaves become more erect so that photosynthesis can run smoothly to increase plant growth [9].

The function was to maintain soil moisture so that the water content in the soil was maintained. This was very beneficial for plants to be resistant to environments that have little water or dry [3]. The other Si’s role was to protect plants from unfavorable conditions [3]. The use of Si functions in strengthening plant stems, protecting plants from pests, strengthening roots and others[10]. Rice plants that were sufficiently Si were resistant to attack by rice stem borer and rice blast, efficient in capturing sunlight because the leaves are pointing up, efficient in using water and not easy to fall because they have strong stems [11].

Based on the things mentioned above, this study was conducted to determine the anatomical response of leaves and agronomic performance of several rice varieties to the application of Silica fertilizer.

2. Material and methods

The study was conducted in the first rainy season of 2018 (November 2017 to March 2018) in Semanu, Gunungkidul, Indonesia. The study was arranged in factorial, the first factor was 4 rice varieties (V1 = Inpari blast, V2 = Inpari 42, V3 = Ciherang, and V4 = Inpari 33), the second factor is the treatment of Silica fertilizer dosage (P1 = 250 kg / ha of Silica fertilizer; P2 = 500 kg / ha of Silica fertilizer, P3 = 1,000kg / ha of husk ash, and P4 = 750 kg / ha of Silica fertilizer). Observing agronomic traits including plant height, number of productive tillers, number of filled grains per panicle, and productivity). In addition to observing agronomic traits, microscopic observations of leaf anatomy were also carried out with thick upper and lower epidermal cells, cuticles in the upper and lower epidermis, mesophyll thickness in major and minor intervenous regions.

The method of slicing rice leaves was using a freehand section, then a semi-permanent preparation made with safranin drops. The microscope used is the BOECO BM-180, using the Optilab Advance Plus version made by miconos. Anatomical parameters of the leaves measured included thickness of upper and lower epidermal cells, cuticles in the upper and lower epidermis, mesophyll thickness in major and minor intervenous regions. Data obtained both agronomic character data and leaf anatomy data were analyzed for variance (ANOVA = Analysis of variance) and Duncan's advanced test using SAS version 9.2.

3. Results and discussion

3.1. Plant height, number of productive tillers, number of filled grains per panicle and productivity

The results of analysis of variance, in general, showed that the application of Silica nutrients had a significant influence on the growth of rice plants in the treatment of variety (V), and the interaction of
varieties and application of Silica fertilizer (VP); but it was not evident in the treatment of Silica fertilizer (P) (Table 1). Varieties have a significant effect on plant height, number of productive tillers, number of filled grains per panicle and productivity. Inpari42 and Ciherang varieties provide the highest productivity. Inpari blast (V1) and Inpari 33 (V4) varieties were less responsive to the application of Silica fertilizer, seen from low productivity both in the treatment of the variety itself and the interaction of varieties and doses of Silica fertilizer. Interaction variety and dosage treatment of Silica fertilizer, the highest was achieved by V2P4, namely interaction of Inpari 42 (V2) varieties at Silica doses of 750 kg/ha (P4) and followed by V3P4 (Ciherang interactions and 750 kg/ha of Silica fertilizer). Based on the agronomic performance, it appears that the provision of 750 kg Silica fertilizer/ha can increase rice growth and production. This is in line with opinion [6], the decrease in Si available in the soil for plants was likely to be closely related to the decline in rice crop productivity. The movement of silica elements out of the paddy fields due to crop yields causes the process of decreasing the silica content available in the soil [12].

Si’s role in increasing the growth and production of rice is caused by the improved photosynthesis system because the leaves become more erect so that photosynthesis can run smoothly to increase plant growth [9]. Besides increasing rice yield, silica can also increase nutrient availability (N, P, K, Ca, Mg, S, Zn), reduce nutrient toxicity (Fe, Mn, P, Al), and minimize biotic and abiotic stress in plants [4]. Increased rice productivity due to the provision of Silica fertilizer was supported by data that the productivity of Cihergar rice varieties without the application of Silica fertilizer on rainfed land in Gunungkidul provides productivity of 7.57 tons/ha [13], lower than the results of this study (Table 1), namely 7.99 tons/ha (treatment of Ciherang variety = V3) and 8.55 tons/ha (Ciherang variety against 750 kg/ha of V3P4 Silica fertilizer). In 1955 Si fertilizer was first given in Japan as much as 1.5 - 2 tons/ha with rice yields increasing by 5 -15% [14]. The highest productivity was achieved by the interaction of Inpari 42 (V2) with 750 kg/ha of Silica fertilizer (V2P4) which was 9.69 tons/ha Harvested Dry Grain.

The application of 1000 kg/ha husk ash as a source of Silica elements provides lower productivity than the provision of Silica Granule fertilizer at a dose of 750 kg/ha. Allegedly the dose of husk ash given was not right. One source of natural silica that can be easily obtained is rice husk. Rice husk was a by-product of the rice milling industry which has a heavy portion of 20% of the rice yield [15]. This rice husk contains 13 to 29% inorganic components and 87 to 97% organic components such as cellulose and lignin. From 13 to 29% of the inorganic component there is a silica content of 87% to 97% [16] so that from this large silica content rice husk has a great potential to be used as organic fertilizer which can increase rice production and rice strength from abiotic or biotic stress [17].

3.2. Leaf anatomy
Anatomical performance of the leaf (upper epidermis, cuticle in the upper epidermis, mesophyll, lower epidermis, and cuticle in the lower epidermis) is presented in Figure 1 (major intervenous) and figure 2 (minor intervenous). From the appearance of the leaf anatomy, the thickness of the upper epidermis, the thickness of the cuticle in the upper epidermis mesophyll/width of the major and minor intervenous leaves, lower epidermis, and cuticles in the lower epidermis. The results of the analysis of variance of the thickness parameters of the epidermis, cuticle, and width of major intervenous and minor intervenous widths are presented in Table 2 and Table 3. The interaction between varieties and dosages of Silica fertilizer showed a significant effect on the thickness of the upper epidermis (tEa), thickness of the upper cuticle (tkA); thick lower epidermis (tEb); thickness of the lower cuticle (tKB) in both the major and minor intervenous leaves (Table 2 and Table 3).

Differences in leaf anatomy were quantified by cuticle thickness, epidermis thickness, and leaf width according to the genetic potential of each variety and their response to the administration of Silica fertilizer. In general, from Table 2 and Table 3 it appears that the application of 750 kg Silica fertilizer/ha to the four varieties shows better leaf anatomy than the other treatments. The anatomical performance of these leaves is linear with high productivity because the application of Silica fertilizer can prevent/reduce pest and disease attacks. [18] states that Silica plays a role in reducing the level of
pest and disease attacks through two mechanisms, which are mechanical barriers and physiological mechanisms in increasing resistance to pests and diseases. A silica layer with a thickness of 2.5 μm under the cuticle produces a double layer of cuticle-silicon that can inhibit or delay pest penetration [19].

Table 1. The results of the analysis of variability in agronomic traits (plant height, number of productive tillers, number of filled grain per panicle and productivity) of several rice varieties on the application of Silica fertilizer

| Variety | V1 = Inpari blast ; V2 = Inpari 42 ; V3 = Cilherang; V4 = Inpari 33 | Plant height(cm) | Number of productive tillers | Number of filled grain per panicle | Productivity (ton/ha Harvested Dry Grain) |
|---------|-----------------------------------------------------------------|------------------|-----------------------------|-----------------------------------|------------------------------------------|
| V1      | 99.19 b                                                         | 11.25 a          | 71.68 c                     | 6.08 c                             |
| V2      | 90.72 c                                                         | 11.94 b          | 99.56 a                     | 7.88 a                             |
| V3      | 103.81 a                                                        | 13.25 ab         | 85.04 b                     | 7.99 a                             |
| V4      | 92.69 c                                                         | 13.89 ab         | 66.00 c                     | 6.87 b                             |

Dosage of Silika ; P1 = 250 kg/ha; P2 = 500 kg/ha; P3 = 1000kg/ha abusekam; P4=750 kg/ha

| Variety | Treatment | Plant height(cm) | Number of productive tillers | Number of filled grain per panicle | Productivity (ton/ha Harvested Dry Grain) |
|---------|-----------|------------------|-----------------------------|-----------------------------------|------------------------------------------|
| V1      | V1P1      | 96.67 a-e        | 16.11 ab                    | 82.27 a-d                         | 5.43 f                                   |
|         | V1P2      | 101.22 a-c       | 18.67 a                     | 64.45 b-d                         | 6.94 d-f                                 |
|         | V1P3      | 104.66 a         | 12.11b-d                    | 75.39 b-d                         | 6.14 ef                                  |
|         | V1P4      | 95.22 c-e        | 14.11 ad                    | 64.61 b-d                         | 5.75 f                                   |
| V2      | V2P1      | 87.78 e-g        | 10.78 cd                    | 111.78 a                          | 7.30 be                                  |
|         | V2P2      | 86.33 fg         | 11.33b-d                    | 91.33 a-c                         | 6.74d-f                                  |
|         | V2P3      | 90.67 d-g        | 9.67 d                      | 89.22 a-c                         | 7.79 b-d                                 |
|         | V2P4      | 98.11 a-d        | 16.0 a-c                    | 105.89 a                          | 9.69 a                                   |
| V3      | V3P1      | 104.78 a         | 12.56 b-d                   | 82.3 a-d                          | 8.1 b-d                                  |
|         | V3P2      | 101.89 a-c       | 13.0 b-d                    | 89.50 a-c                         | 8.35 a-c                                 |
|         | V3P3      | 104.78 a         | 12.00 b-d                   | 94.05 ab                          | 6.94 c-f                                 |
|         | V3P4      | 103.78 ab        | 15.44 a-c                   | 74.28 b-d                         | 8.55 ab                                  |
| V4      | V4P1      | 94.11 c-f        | 13.56 a-d                   | 56.28 d                           | 7.81 b-d                                 |
|         | V4P2      | 98.11 a-d        | 14.22 a-d                   | 75.333 b-d                        | 7.44 b-e                                 |
|         | V4P3      | 93.44 c-f        | 15.33 a-c                   | 62.55 cd                          | 6.65 d-f                                 |
|         | V4P4      | 85.11 g          | 12.44 b-d                   | 69.83 b-d                         | 5.58 f                                   |

CV (%)) 4.55 19.72 10.38 10.88

Note: Numbers followed by the same letters in the same row and column were not significantly different according to Duncan's Multiple Range Test at 5%
Table 2. Results of analysis of variance in cuticle thickness, epidermis and width of major intervenous leaves of several varieties on the application of Silica fertilizer.

| Treatment | Thick upper cuticle | Thick upper epidermis | Width of major intervenous leaves | Thick bottom epidermis | Thick cuticle down |
|-----------|---------------------|-----------------------|-----------------------------------|------------------------|-------------------|
| **Variety** |                     |                       |                                   |                        |                   |
| V1        | 5.39 a              | 5.77 a                | 193.79 a                          | 6.40 ab                | 4.47 a            |
| V2        | 4.73 ab             | 5.46 a                | 173.04 b                          | 5.35 c                 | 4.21 a            |
| V3        | 5.37 a              | 5.75 a                | 150.49 c                          | 5.78 bc                | 4.48 a            |
| V4        | 4.26 b              | 5.28 a                | 184.05 ab                         | 6.67a                  | 4.33 a            |
| **Dosage of Silika** |               |                       |                                   |                        |                   |
| P1        | 4.68 a              | 5.59 ab               | 182.41 a                          | 6.10a                  | 4.31 a            |
| P2        | 4.78 a              | 6.07 a                | 167.14 b                          | 6.06a                  | 4.08 a            |
| P3        | 5.08 a              | 5.30 b                | 172.69 ab                         | 5.81 a                 | 4.45 a            |
| P4        | 5.21 a              | 5.30 b                | 179.13 ab                         | 6.22 a                 | 4.65 a            |
| **Interaction of Variety and Dosage of Silica fertilizer** |             |                       |                                   |                        |                   |
| V1P1      | 4.85 a-d            | 5.57 a-c              | 219.55 a                          | 5.98 b-d               | 3.13 b            |
| V1P2      | 5.48 ab             | 6.36 ab               | 160.93 d-f                        | 7.55 ab                | 4.38 ab           |
| V1P3      | 5.50 ab             | 5.55 a-c              | 187.16 b-d                        | 5.59 cd                | 4.29 ab           |
| V1P4      | 5.72 a              | 5.59 a-c              | 207.54 ab                         | 6.48 bc                | 5.25 a            |
| V2P1      | 3.90 cd             | 5.74 a-c              | 175.42 c-e                        | 5.69 cd                | 4.14 ab           |
| V2P2      | 4.55 a-d            | 6.59 a                | 168.10 c-f                        | 5.65 cd                | 3.13 b            |
| V2P3      | 5.12 a-d            | 4.61 c                | 188.67 b-d                        | 5.49 cd                | 5.12 a            |
| V2P4      | 5.36 a-c            | 4.91 bc               | 159.95 d-f                        | 4.58 d                 | 4.43 a            |
| V3P1      | 5.38 a-c            | 6.16 a-c              | 150.63 ef                         | 6.52 bc                | 4.49 a            |
| V3P2      | 5.32 a-c            | 5.93 a-c              | 145.64 ef                         | 5.45 cd                | 4.76 a            |
| V3P3      | 5.12 a-d            | 5.95 a-c              | 163.66 d-f                        | 5.48 cd                | 4.29 ab           |
| V3P4      | 5.65 a              | 4.97 a-c              | 142.02 f                          | 5.65 cd                | 4.39 ab           |
| V4P1      | 4.59 a-d            | 4.90 bc               | 184.04 b-d                        | 5.21 b-d               | 4.66 a            |
| V4P2      | 3.76 d              | 5.39 a-c              | 193.89 a-c                        | 5.58 cd                | 4.06 ab           |
| V4P3      | 4.56 a-d            | 5.1 a-c               | 191.28 ef                         | 6.68 a-c               | 4.08 ab           |
| V4P4      | 4.12 b-d            | 5.72 a-c              | 207.01 ab                         | 8.16 a                 | 4.51 a            |
| **CV (%)** | 20.39               | 19.78                 | 11.79                             | 18.46                  | 19.61             |

Note: Numbers followed by the same letters in the same row and column were not significantly different according to Duncan's Multiple Range Test at 5%.
Dosage of Silika Fertilizer

| Variety     | P1 = 250 kg/ha Silika | P2 = 500 kg/ha Silika | P3 = 1000 kg/ha Silika | P4 = 750 kg/ha Silika |
|-------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Inpariblas  | ![Image]              | ![Image]              | ![Image]              | ![Image]              |
| Inpari 42   | ![Image]              | ![Image]              | ![Image]              | ![Image]              |
| Ciherang    | ![Image]              | ![Image]              | ![Image]              | ![Image]              |
| Inpari 33   | ![Image]              | ![Image]              | ![Image]              | ![Image]              |

**Figure 1.** Anatomy of the major intervenous leaves of several rice varieties on the administration of Silica fertilizer
| Variety      | Intervena Minor Dosage of Silika Fertilizer |
|-------------|---------------------------------------------|
| Inpariblas  | P1 = 250 kg/ha Silika                       |
|             | P2 = 500 kg/ha Silika                       |
|             | P3 = 1000 kg/ha abusekam Silika             |
|             | P4 = 750 kg/ha Silika                       |
| Inpari 42   |                                             |
| Ciherang    |                                             |
| Inpari 33   |                                             |

**Figure 2.** Anatomy of the minor intervenous leaves of several rice varieties on the administration of Silica fertilizer.
### Table 3. Results of analysis of variance in cuticle thickness, epidermis and width of minor intervenous leaves of several varieties on the application of Silica fertilizer

| Treatment | Thick upper cuticle | Thick upper epidermis | Width of major intervenous leaves | Thick bottom epidermis | Thick cuticle down |
|-----------|---------------------|-----------------------|-----------------------------------|------------------------|--------------------|
| Variety   | V1 = Inpari blast ; V2 = Inpari 42; V3 = Ciherang; V4 = Inpari 33 |                       |                                   |                        |                    |
|           | V1                   | 5.65 a                | 4.56 bc                           | 100.61 a               | 5.0 a              |
|           | V2                   | 4.12 b                | 4.38 c                            | 94.10 b                | 5.42 a              |
|           | V3                   | 4.19 b                | 5.01 ab                           | 87.37 c                | 5.51 a              |
|           | V4                   | 4.21 b                | 5.23 a                            | 97.54 ab               | 5.41 a              |
| Dosage of Silica |                     |                       |                                   |                        |                    |
|           | P1                   | 4.52 ab               | 4.56 bc                           | 101.26 a               | 5.47 ab             |
|           | P2                   | 4.84 a                | 5.37 a                            | 91.54 b                | 5.57 a              |
|           | P3                   | 4.00 b                | 4.67 b                            | 94.25 b                | 4.89 b              |
|           | P4                   | 4.81 a                | 4.58 b                            | 91.66 b                | 5.41 ab             |
| Interaction of Variety and Dosage of Silica fertilizer |                     |                       |                                   |                        |                    |
|           | V1P1                 | 5.32 ab               | 3.84 d                            | 120.11 a               | 4.49 cd             |
|           | V1P2                 | 6.32 a                | 5.25 a-c                          | 88.70 de               | 5.17 b-d            |
|           | V1P3                 | 4.51 bc               | 4.67 a-d                          | 95.46 b-d              | 5.12 b-d            |
|           | V1P4                 | 6.44 a                | 4.48 a-d                          | 98.15 b-d              | 5.22 b-d            |
|           | V2P1                 | 3.48 c                | 4.38 a-d                          | 97.64 b-d              | 5.67 a-c            |
|           | V2P2                 | 4.83 a-c              | 4.76 a-d                          | 87.63 de               | 5.21 b-d            |
|           | V2P3                 | 3.43 c                | 3.98 cd                           | 100.68 bc              | 5.85 a-c            |
|           | V2P4                 | 4.75 a-c              | 4.40 a-d                          | 90.83 cd               | 4.95 b-d            |
|           | V3P1                 | 3.79 bc               | 5.41ab                            | 88.55 de               | 5.50 bc             |
|           | V3P2                 | 4.36 bc               | 5.74 a                            | 92.92 b-d              | 6.82 a              |
|           | V3P3                 | 4.72 a-c              | 4.71 a-d                          | 88.52 de               | 4.11 d              |
|           | V3P4                 | 3.89 bc               | 4.18 b-d                          | 79.49 e                | 5.61 a-c            |
|           | V4P1                 | 5.49 ab               | 4.62 a-d                          | 102.74 b               | 6.20 ab             |
|           | V4P2                 | 3.84 bc               | 5.73 a                            | 96.91 b-d              | 5.08 b-d            |
|           | V4P3                 | 3.35 c                | 5.29 a-c                          | 96.36 b-d              | 4.50 cd             |
|           | V4P4                 | 4.14 bc               | 5.26 a-c                          | 98.15 b-d              | 5.84 a-c            |

| CV (%) | 26.49 | 19.58 | 7.88 | 19.46 |

Note: Numbers followed by the same letters in the same row and column were not significantly different according to Duncan's Multiple Range Test at 5%.

**4. Conclusion**

The application of silica fertilizer increases rice growth and productivity and increases the thickness of the epidermis and upper and lower cuticles in both major and minor intervenes. The highest productivity was achieved by Inpari42 varieties in the application of 750 kg/ha Silica fertilizer (9.69 ton/ha).
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