Effect of Plant Growth Regulators and Planting Density against Viral Infection and the Production from Bulbs of True Shallot Seed in the Highlands

N Gunaeni*, W Adiyoga, R Rosliani and I Sulastrini
Indonesia Vegetable Research Institute
Jl. Tangkuban Perahu No. 517 Lembang - West java

*nenigunaeni@yahoo.com

Abstract. In general shallots are cultivated using bulbs (vegetatively). The problem is the cost of controlling bulbs is quite high, which is around 40% of the total. One way to increase the productivity of shallots is by using healthy plant material through using of True Shallot Seeds (TSS). TSS is one of the generative propagation methods of shallots from seed. The research was carried out at the Indonesia Vegetable Research Institute in Lembang (plateau 1250 asl). Time of implementation from August 2017 to April 2018. Research is conducted in the Field and laboratory. The experimental design used is a factorial randomized completely block design with two factors repeated 3 times. The first factor type of Plant Growth Regulators consists of: A.1 = BAP concentration of 50 ppm, A.2 = NAA concentration of 50 ppm, A.3 = GA3 concentration of 50 ppm, A.4 = Control. The second factor is planting density: B.1 = Planting density of 5 gram of seeds / m^2, B.2 = Planting density of 7 gram of seeds / m^2, B.3 = Planting density of 9 gram of seeds / m^2. The results of the study show that: (1). The giving of Plant Growth Regulators (BAP, NAA and GA3) does not affect the germination of True Shallot Seed and plant growth, the number of living plants and tuber yields in the field. (2). planting density affects plant growth in the field and greenhouse area 5 gram of seeds and 7 gram of seeds / m^2 land area. (3). The results of the Elisa test on leaf samples not detected SYSV, OYDV and LYSV viruses. (4). Planting density of 7 gram of seeds / m^2 land area has a good effect on tuber yields in the field. The number of cloves produced in the field is 1 to 5 clove.

Keywords: Allium cepa var. Ascalonicum, plant growth regulators, planting density, True Shallot Seed

1. Introduction
Shallot (Allium cepa var. Ascalonicum) is a priority vegetable in dry land farming because it has high economic value. The production of shallots is relatively high in several provinces, namely Central Java, East Java, West Java and West Nusa Tenggara respectively 29.63%, 24.41%, 11.15% and 14.15%. The harvest area of shallot in Indonesia in 2018 was around 142.547 ha with a production of 1.206.266 tons with an average yield of around 8.46 t / ha [1]. The average production is still low as compared to the potential results of Indonesia Vegetable Research Institute on local varieties which can reach 15 t / ha [2]. The cause of low shallots production is due to the difficulty of getting good quality seed bulbs without disease especially virus. Viral diseases are one of the important diseases because it can reduce
the quality and quantity of shallots. Viruses are obligate parasites that can multiply after entering into living cells of the host plant and causing plant anatomical changes and external symptoms such as mosaics, curly leaves, curling and rugosa. The way virus enters into the host plant depends on the type of virus that attacks the plant. Losses due to viral diseases in the field depend on the symptoms seen on the shallots plant. Symptoms of viral attack on shallot plants vary depending on the type of virus, plant age, host, vector and environmental factors. According to several studies, used of healthy garlic seed bulbs will increase tuber yield more than 100% compared to seed tubers infected with the virus can reduce tuber weight results 66-216% and tuber diameter 13-51% [3-4].

There have been reported several Potyvirus that attack shallot plants namely Onion Yellow Drawf Virus (OYDV), Shallot Yellow Stripe Virus (SYSV), and Leek Yellow Stripe Virus (LYSV) [5-9]. The attacks of LYSV, OYDV and IYSV (Iris Yellow Spot Virus) can cause yield losses of respectively 74%, 54% and 60%. Previous studies reported that virus free tuber weights at garlic plants have been shown to be 32–216% higher than plants infected with the virus [10]. While other study reported the weight of garlic tubers infected with the virus decreased 78% [4]. OYDV, SLV (Shallot Latent Virus), and LYSV viruses can be transmitted by insect vectors *Myzus persicae*, *M. ascalonicum*, and *Aphis faba*. The efficiency of the distribution of insect vectors *M. persicae*, *M. craccivora*, *A. gosspini* and *A. pisum* were respectively 66.7%, 60.0%, 33.30% and 8.30% for OYDV-G and 63.60%, 40%, 20% and 20% for LYSV-G [11-12]. In the tropics, the vector will always be active and breed all the time as a result of the host not having a rest period. These viruses can cause tuber infection because shallot is always propagated vegetatively [13-14], so that the virus develops and accumulates in the tuber eventually carried by the seeds. The insect vector plays an important role because it can transmit viral diseases by inserting stylets on diseased plants, which then stab again into other healthy plants. Viruses that have been found in an area, maybe there are also in other areas or vice versa, this is because the virus can spread because there are sources of infection, vectors and vector activity.

In general shallots are cultivated using seed bulbs (vegetatively). The problem is the cost of controlling seed bulbs is quite high, which is around 40% of the total production cost [15]. One way to increase the productivity of shallots is by using healthy plant material through the use of True Shallot Seeds (TSS). TSS is one method of generative propagation of red onion seeds in the form of seeds [2, 16]. Using of TSS when viewed economically is very beneficial because it can increase yields by up to two times compared to the use of traditional tuber seeds [17]. However, Gunaeni et al. [18] explained that if virus particles are found in plant tissue, the seeds will be carried over to the next crop.

Various efforts to improve the productivity and quality of shallot plants can be done by using growth regulators that play a role in stimulating plant growth and yield as well as the use of appropriate growing media. Growth regulators are one of the substances to stimulate plant growth and increase crop yield. Auxin, carbohydrates and nitrogen in plant material can induce the formation of roots. Some auxin compounds which are widely used for rooting are Indole Acetic Acid (IAA), Indole Buteric Acid (IBA) and Naphthalene Acetic Acid (NAA) [19]. Generally, growth regulators applied through plant leaves or canopies, so that plants can absorb it quickly. In general, hormones or growth substances are chemicals that are made in certain plant parts that affect plant growth and development. Although each plant can produce its own growth regulators, using of growth regulators from the environment can stimulate metabolic processes in plant growth and development [20]. Based on this, the purpose of this study was to determine the effect of growth regulators and plant density on shallot production from shallot seeds and their effect on viral infections in the highlands.

2. Materials and Methods
The research was carried out at the Indonesia Vegetable Research Institute in Lembang (plateau 1250 asl). Time of implementation from August 2017 to April 2018. Research is carried out in the field, greenhouse and in the laboratory.

The experimental design used is factorial randomized completely block design with two factors that are repeated 3 times. The first factor (A) used was types of plant growth regulators consists of (A.1) BAP concentration of 50 ppm, (A.2) NAA concentration of 50 ppm, (A.3) GA3 concentration of 50
ppm, (A.4.) control. The second factor (B) used was planting density consisted of (B.1.) planting density of 5 grams of seeds/ m², (B.2.) planting density of 7 grams of seeds/ m², (B.3.) planting density of 9 grams of seeds/ m².

There were 12 treatment combinations that were repeated 3 times with the total experimental unit being 36 combinations. The shallot variety from TSS used is Trisula. TSS seeds treated by soaking with growth regulators were weighed and packed in gauze bags according to treatment. Seeds in gauze are placed in growth regulators for 1 hour and dried for 1 night before sowing. The nursery land is solarized by pouring water into the soil to be evenly spread and covering it using clear plastic (Polyethylene). Bed soil temperature is measured until it reaches 52 °C. When the soil temperature reaches 52 °C, Polyethylene plastic is opened and a hole is made which is coated with a burlap sacke. Seedling media (manure + charcoal husk + soil + dolomite in a ratio of 1: 1: 1: 1) spread over burlap sacke and made beds as high as 10 cm with a size of 1 m². TSS seeds on spread evenly in beds then the pit is covered with seedbed media at the top of the beds. Watering is done evenly with low pressure so that the seeds do not fall apart and the beds are covered by using burlap sacks to maintain moisture until the seeds appear to grow. Burlap sacks are opened when the seedlings have grown evenly. NPK pearl fertilizer (16:16:16) with a dose of 10 gram of seeds / m² is given twice with evenly sprinkled on all beds at the age of 30 and 60 days after seedling. Pest control is done only when pest attacks and adapted to the type of pesticide. Maintenance is carried out in accordance with plant conditions.

Variables observed:
1. Percentage of seeds that grow
2. Plant growth (plant height, number of leaves, and number of tillers). Observations were made 40 days after planting at one week intervals. Plant height is measured from ground level to the highest shoots.
3. Number of living plants
4. Test of Serology of the Elisa method. Serology testing was carried out using the Elisa method directly using the Onion Yellow Dwarf (OYDV) antiserum and Shallot Yellow Stripe Virus (SYSV) as follows: IgG (Primediagnostic, the Netherlands) was dissolved by coating buffer at a concentration of 1: 1,000 (w / v). Each plate hole is filled with 100 mL of solution. Plate was incubated at 37 °C for 4 hours. The plate was then washed 0.02 M PBS-T three times. The antigen samples were crushed with a concentration of 1: 10 (b / v) with 0.02 M PBS-T containing 2% PVP and 0.2% ovalbumin inserted into the 100 µl plate hole. The plate containing the sample was incubated at 4 °C for 1 night. The next day the plate was washed with 0.02 M PBS-T six times. Enzim conjugate (Primediagnostic, the Netherlands) dissolved in 0.02 PBS-T with a concentration of 1: 1,000. Each plate hole is filled with 100µl. Then the plate was incubated at 37 °C for 3 hours. The plate was washed with 0.02 M PBS-T six times. PNP substrate 1 mg / ml in diethanolamine buffer was added and each plate hole was filled as much as 150 µl. Absorbance was measured using Elisa Reader (Bio-Rad Model 550) at A405 nm after incubating for 30-60 minutes.
5. Tuber yields (number of tubers, tuber weight, tuber grading).

Data were analyzed by Analysis of Variance (Anova). If the F test is real in the treatment, then there is a difference between the test and control plants, then a further test is carried out using the middle value test Duncan's Multiple Distance (DMRT).

3. Results and Discussion

3.1. Percentage of seed germination that grows

The soaking of shallot seeds from TSS with of Plant Growth Regulators at a concentration of 50 ppm resulted in different growth of germination in each treatment. The germination test was carried out to determine the seed growth from the best treatment. The observations can be seen in (Table 1). Based on (Table 1) above, it can be seen that the best germination in the control treatment was significantly
different from the treatment of NAA and GA3, while the inter-control treatment with BAP or BAP with NAA and GA3 was different but not too significant. The treatment of BAP, NAA and GA3 does not affect the germination, this may be due to a balance between exogenous growth regulating substances and endogenous hormones from plants. [21] states that the application of growth regulators with high concentrations can inhibit seed development, because the process of cell division was disrupted. The work of growth regulators on plant tissue influenced plant physiology and morphology.

**Table 1.** Average of shallot seeds germination test of the shallot seeds.

| Treatment | Germination Rate (%) |
|-----------|----------------------|
| BAP       | 39.00 ab             |
| NAA       | 30.60 b              |
| GA3       | 30.60 b              |
| Control   | 51.20 a              |

3.2. Effect of Plant Growth Regulators on shallot plant growth
The effect of Plant Growth Regulators treatment and planting density on shallot seeds from TSS from growth in plant height, number of tillers and number of leaves in the field can be seen in (Figure 1 and 2).

3.2.1. Plant height growth. Growth of plant height in the field at the age of 75 days after planting (Figure 1) shows NAA treatment and planting density of 5 grams of seeds / m² higher than other treatments and controls. This is because NAA is a growth regulating substance from the auxin group and at certain concentrations it functions as root initiation and plant stem growth. These results are the same as those stated by [22] stated that plant height increment is a process caused by auxin activity because one of the roles of auxin is to support cell elongation. Auxin in low concentrations can stimulate the enlargement and extension of cells after cell division is stimulated by cytokines, but if the concentration of auxin used is too high, it will inhibit cell elongation.

**Figure 1.** Effect of plant growth regulators application and planting density on the height of shallots in the field.
3.2.2. Number of leaves. The treatment of Plant Growth Regulators and planting density on the growth of the number of leaves at the age of 61 to 75 in the field was not significantly different (Figure 2). This is as stated by [22] that the higher addition of NAA tends to cause the average number of leaves is lower than treatment without NAA. This result due to an increase in NAA concentration which can inhibit leaf growth, while the use of high concentrations of cytokinin combined with low concentration of auxin is very important in leaf formation.

![Graph showing number of leaves over time](image)

**Description:** (1). BAP + 5 g of seeds/m², (2). BAP + 7 g of seeds / m², (3). BAP + 9 g of seeds/m², (4). NAA + 5 g of seeds/m²; (5). NAA + 7 g of seeds/m², (6). NAA + 9 g of /m², (7). GA3 + 5 g of seeds/m², (8). GA3 + 7 g of seeds/m², (9). GA3 + 9 g of seeds /m², (10). Control + 5 g of seeds/m², (11). Control + 7 g of seeds/m², (12). Control + 9 g seed/m².

**Figure 2.** Effect of Plant Growth Regulators and planting density on the number of leaves of shallots in the field.

3.2.3. Number of tillers. The influence of Plant Growth Regulators on the growth of the most number of tillers in the field was found in treatment 4, namely NAA with a density of 5 g / m² (Figure 3). This is in accordance with the theory that growth regulators can increase source-sink translocation and encourage photo-assimilate translocation that helps in the formation of flowers, development of seeds and capsules effectively and ultimately increases plant productivity. This happens because growth regulators can improve physiological efficiency including photosynthetic ability and can increase the distribution of assimilates from source to plant sinks [24,25].

![Graph showing number of tillers over time](image)

**3.3. Number of living plants**

Observation of the number of living plants carried out at harvest time, after the seeds sown grow and survive forming tubers. The effect of Plant Growth Regulators and planting density on growing plants can be seen in (Figure 4). TSS sown or planted on 1 m² of land with a volume of 5 gram containing 1600 seeds, 7 gram containing 2240 seeds and 9 gram containing 2880 seeds, Plants from TSS that survive until harvest vary depending on Plant Growth Regulators treatment and planting density. In the treatment field GA3 + 9 g of seeds / m² and NAA + 7 gr of seeds / m² more than other treatments and controls. The plants that lived were only 20.83% and 26.03% of the seeds sown. The plants that lived were only 38.82%, 32.05 and 24.16% of the total seeds sown. The number of plants that live is more proportional to the number of seeds sown, because many seeds are sown per m² area, the more likely the plants live. The number of plants that live compared to the number of seeds sown is very low, this may be due to the testing data of seed sprout power without of Plant Growth Regulators.
only growing by 50%. Besides that, too dense and dense plants cause more disease compared to wide-range plants. It can be concluded that the fewer the number of plants per unit area or the wider the spacing, then the plant growth will be better than plants planted more tightly. These results are in line with previous studies that the higher the density of plants, the slower plant growth [26]. It seems that Plant Growth Regulators does not affect the number of living plants, the effect is the planting density of 7 g and 9 g / m².

**Figure 3.** Effect of Plant Growth Regulators and planting density on the number of tillers shallots on the field.

**Description:** (1). BAP + 5 g of seeds/m², (2). BAP + 7 g of seeds/m², (3). BAP + 9 g of seeds/m², (4). NAA + 5 g of seeds/m²; (5). NAA + 7 g of seeds/m², (6). NAA + 9 g of seeds/m², (7). GA3 + 5 g of seeds/m², (8). GA3 + 7 g of seeds/m², (9). GA3 + 9 g of seeds/m², (10). Control + 5 g of seeds/m², (11). Control + 7 g of seeds/m², (12). Control + 9 g of seed/m².

**Figure 4.** Effect of Plant Growth Regulators and planting density on the living plants of shallots in the field.

**Description:** (1). BAP + 5 g of seeds/m², (2). BAP + 7 g of seeds/m², (3). BAP + 9 g of seeds/m², (4). NAA + 5 g of seeds/m²; (5). NAA + 7 g of seeds/m², (6). NAA + 9 g of seeds/m², (7). GA3 + 5 g of seeds/m², (8). GA3 + 7 g of seeds/m², (9). GA3 + 9 g of seeds/m², (10). Control + 5 g of seeds/m², (11). Control + 7 g of seeds/m², (12). Control + 9 g of seed/m².
3.4. Test the Serology of Elisa method

The shallot leaf samples were taken at plant age 12 week after planting and serological tested using the indirect method using OYDV and LYSV antiserum. Elisa test results on samples from the field and greenhouses (Tables 2) can be seen that there is no yellow color in the sample. In Elisa's reading, the sample can be said to be positive if the results obtained are twice negative control. Elisa's reading proves that the plants were not infected with the OYDV or SYSV viruses. This situation indicates that the plants and seedlings of onion may have a virus other than OYDV and SYSV or have been tolerant with the OYDV and SYSV viruses. The use of TSS seeds also has several advantages compared to the use of seed bulbs, namely TSS can produce healthier plants because TSS is free of disease pathogens, and produces better quality and better tuber [16,27-29]. Viral diseases are one of the obstacles in increasing the production of shallots (A. cepa L.). The OYDV virus onion when joining SLV and SYSV shows more severe symptoms. Symptoms of the virus are generally chlorosis, intermittent yellow stripes, leaves with green stripes and leaf size to be relatively smaller [17,30, 31].

Symptoms will not appear along with increasing age of the plant [32]. [17] reported that OYDV and SYSV infections could reduce the weight of shallot bulbs by 4.65% in Bima Curut varieties and the Philippines, whereas according to [31] loss of yield due to OYDV infection can reduce tuber weight by 21.5%. The study was conducted to detect the main viruses of shallots from planting in the field using the ELISA method. Accurate virus detection methods determine the results of disease monitoring in the field to obtain virus-free seeds. Serological techniques such as ELISA are sophisticated techniques that are promising for the detection and identification of plant vegetative [33,34]. Serological techniques can be widely accepted by users, especially for detecting large quantities of viruses. The material tested can be directly in the form of sick plant extract without having to isolate the pathogen first [36]. Should be stated that the result of ELISA test was presented in (Table 2).

3.5. Tuber yields (number of tubers, tuber weight, tuber grading)

The effect of Plant Growth Regulators and planting density on the yield of shallot bulbs in the field is distinguished by the weight of wet and dry tubers and the number of tubing tubers (Figure 5, 6 and 7).

![Figure 5](image_url)

**Description:** (1). BAP + 5  g of seeds/m², (2). BAP + 7  g of seeds/m², (3). BAP + 9  g of seeds/m², (4). NAA + 5  g of seeds/m²; (5). NAA + 7  g of seeds/m², (6). NAA + 9  g of seeds/m², (7). GA3 + 5  g of seeds/m², (8). GA3 + 7  g of seeds/m², (9). GA3 + 9  g of seeds/m², (10). Control + 5  g of seeds/m², (11). Control + 7  g of seeds/m², (12). Control + 9  g of seed/m².

**Figure 5.** Effect of Plant Growth Regulators and planting density on shallot tuber weight in the field per m².
Table 2. Elisa test results of OYDV and SYSV virus with the Indirect Elisa method

| No | Sample | Antiserum |
|----|--------|-----------|
|    |         | SYSV      |          | OYDV   |
|    |         | Visual    | Absorbance | Visual | Absorbance |
| 1  | I.1 (BAP + 5 g of seeds/m²) | - | 0.006 | - | 0.006 |
| 2  | I.2 (BAP + 7 g of seeds/m²) | - | 0.031 | - | 0.031 |
| 3  | I.3 (BAP + 9 g of seeds/m²) | - | 0.040 | - | 0.040 |
| 4  | I.4 (NAA + 5 g of seeds/m²) | - | 0.032 | - | 0.032 |
| 5  | I.5 (NAA + 7 g of seeds/m²) | - | 0.025 | - | 0.025 |
| 6  | I.6 (NAA + 9 g of seeds/m²) | - | 0.033 | - | 0.033 |
| 7  | I.7 (GA3 + 5 gr of seeds/m²) | - | 0.002 | - | 0.011 |
| 8  | I.8 (GA3 + 7 gr of seeds/m²) | - | 0.020 | - | 0.020 |
| 9  | I.9 (GA3 + 9 g of seeds/m²) | - | 0.015 | - | 0.015 |
| 10 | I.10 (Control + 5 g of seeds/m²) | - | 0.011 | - | 0.011 |
| 11 | I.11 (Control + 7 g of seeds/m²) | - | 0.024 | - | 0.024 |
| 12 | I.12 (Control + 9 g of seeds/m²) | - | 0.024 | - | 0.024 |
| 13 | II.1 (BAP + 5 g of seeds/m²) | - | 0.040 | - | 0.102 |
| 14 | II.2 (BAP + 7 g of seeds/m²) | - | 0.012 | - | 0.061 |
| 15 | II.3 (BAP + 9 g of seeds/m²) | - | 0.066 | - | 0.109 |
| 16 | II.4 (NAA + 5 g of seeds/m²) | - | 0.160 | - | 0.097 |
| 17 | II.5 (NAA + 7 g of seeds/m²) | - | 0.072 | - | 0.099 |
| 18 | II.6 (NAA + 9 g of seeds/m²) | - | 0.046 | - | 0.094 |
| 19 | II.7 (GA3 + 5 g of seeds/m²) | - | 0.056 | - | 0.056 |
| 20 | II.8 (GA3 + 7 g of seeds/m²) | - | 0.057 | - | 0.057 |
| 21 | II.9 (GA3 + 9 g of seeds/m²) | - | 0.043 | - | 0.425 |
| 22 | II.10 (Control + 5 g of seeds/m²) | - | 0.157 | - | 0.157 |
| 23 | II.11 (Control + 7 g of seeds/m²) | - | 0.040 | - | 0.040 |
| 24 | II.12 (Control + 9 g of seeds/m²) | - | 0.017 | - | 0.017 |
| 25 | III.1 (BAP + 5 g of seeds/m²) | - | 0.022 | - | 0.022 |
| 26 | III.2 (BAP + 7 g of seeds/m²) | - | 0.040 | - | 0.040 |
| 27 | III.3 (BAP + 9 g of seeds/m²) | - | 0.012 | - | 0.012 |
| 28 | III.4 (NAA + 5 g of seeds/m²) | - | 0.032 | - | 0.033 |
| 29 | III.5 (NAA + 7 g of seeds/m²) | - | 0.029 | - | 0.029 |
| 30 | III.6 (NAA + 9 g of seeds/m²) | - | 0.020 | - | 0.020 |
| 31 | III.7 (GA3 + 5 g of seeds/m²) | - | 0.017 | - | 0.017 |
| 32 | III.8 (GA3 + 7 g of seeds/m²) | - | 0.024 | - | 0.024 |
| 33 | III.9 (GA3 + 9 g of seeds/m²) | - | 0.021 | - | 0.021 |
| 34 | III.10 (Control + 5 g of seeds/m²) | - | 0.026 | - | 0.026 |
| 35 | III.11 (Control + 7 g of seeds/m²) | - | 0.038 | - | 0.038 |
| 36 | III.12 (Control + 9 g of seeds/m²) | - | 0.077 | - | 0.077 |
| 37 | Control (+) | + | 0.305 | + | 0.119 |
| 38 | Control (-) | - | 0.038 | - | 0.054 |

Description: - = negative; + = positively infected with a virus
Figure 6 showed the weight of wet and dry shallot tuber produced in different treatments of Plant Growth Regulators and planting density was seen NAA + 7 gram of seeds / m² (5.90 kg) treatment with dry weight (3.35 kg) or decreased 43.58% higher than other treatments and controls (5.30 kg) with dry weight (2.53 kg) or decreased by 39.90%. The decrease in wet weight to dry weight in the field ranged between 39.90% - 43.58%. The number of cloves formed in the field ranges from 1 - 5 cloves (Figure 7). It was seen that the number of cloves formed was produced by single and highest whistle at treatment GA3 + 9 gram of seeds / m² (408.67 cloves) compared to other treatments and control while the lowest was control + 7 gram of seeds / m². The highest number of 2 whistles was seen in the treatment of GA3 + 9 gram of seeds/ m² (176.56 cloves) compared to other treatments and controls, and the lowest BAP + 5 grams of seeds/ m² (79 cloves).

**Figure 6.** Effect of Plant Growth Regulators and planting density on shallots weight of clove in the field per m².

**Figure 7.** Effect of Plant Growth Regulators and planting density on number of cloves on shallots in the field per m².
4. Conclusion
Application of Plant Growth Regulators (BAP, NAA and GA3) does not affect the germination of True Shallot Seed and plant growth, the number of living plants and tuber yields in the field. Planting density affects plant growth in the field are 5 grams and 7 grams per m² land area. Elisa test results on leaf samples did not detect SYSV, OYDV and LYSV viruses. Planting density of 7 gram of seeds / m² land area has a good effect on tuber yields in the field the number of cloves produced in a single whistle field is up to 5.

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