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Submission date: 29-Jun-2022 10:01AM (UTC+0700)
Submission ID: 1864418878
File name: Application_of_Bacillus_subtilis_on_red_onion_(Allium_ascalonicum_L).pdf (214.45K)
Word count: 2843
Character count: 14931

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To cite this article: A D T Gau *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **921** 012078

Application of *Bacillus subtilis* on red onion (*Allium ascalonicum)*

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Abstract. The increase of public consumption of red onion urge the increased production in order to meet consumer needs. To increase red onion production, it is necessary to formulate cheap and easy technology. This study aims to examine the effect of *Bacillus subtilis* on the growth and production of red onion (*Allium ascalonicum*). The research was conducted at the Laboratory of Plant Diseases, Department of Plant Pests and Diseases and Experimental Fam Land, Faculty of Agriculture, Hasanuddin University Makassar. The study was conducted from February to April 2020. 80-days old red onion were treated with *Bacillus subtilis* bacteria consisting of 4 levels, namely control (p0), 10^4 density (p1), 10^8 density (p2), and 10^{12} density (p3) . The results showed that the application of *Bacillus subtilis* affected the number of leaves, number of stems (tillers), number of bulbs, bulbs diameter (mm), and bulbs wet weight per plant (grams) with the best treatment at a density of 10^8 (p2). The rate of sprouting was not affected by the application of *Bacillus subtilis.*

1. Introduction

Red onion are one of the spice commodities that are used as cooking ingredients [1]. In addition, red onion are also used as herbal medicine in medical therapy [2]. These various benefits make this commodity very important in everyday life. Consumer demand also increases along with the increase in population, so red onion production needs to be increased [3]. To promote the increase of red onion production, it is necessary to apply appropriate cultivation techniques to increase red onion productivity.

Red onion cultivation is carried out vegetatively and generatively by using two seed sources, namely tubers and seeds [4]. The cultivation of red onion using tubers is the most preferred by farmers because it is easier to apply, results in faster growth of shoots and tillers and faster harvest time because it does not need to be sown [5]. The propagation of red onion bulbs can be increased by several ways, namely selecting the size of the seed bulb > 2.5 grams per bulb, adding growth regulators, and microelement nutrients [6]. Selection of good seed is characterized by the bulbs that have broken their dormancy, are healthy, and are of optimal size. The size of the bulbs determines the food reserves that play a role in supporting plant growth and development [7].

The availability of good quality seeds is still an obstacle in the red onion production process, so it is necessary to apply a technology that can be easily applied by farmers [8]. One way that can be done is by giving growth regulators (PGR) to red onion seeds to increase plant metabolism [4]. One of the

PGR used is known as Plant Growt Promoting Rhizobacteria (PGPR) which consists of a collection of root bacteria (Rhizobium, Azotobacter, and Azospirillum) and phosphate solubilizing bacteria (Pseudomonas, Bacillus, and Cerratia) which play a role in improving the nitrogen fixation process, stimulates the growth of fixation bacteria, increases the availability of nutrients, stimulates hormone production, and controls pests and diseases [9]. This alternative technology is used to reduce the use of fertilizers and pesticides so as to encourage the development of environmentally friendly agriculture [10].

Several researchers have analyzed the effect of PGPR on plant growth and production, A'yun et al. [11] stated that giving PGPR with a concentration of 10 ml/L in pepper can increase the growth and yield of these plants; Iswati [12] shows that giving PGPR at a concentration of 12.5 ml / L has a significant effect on plant height and root length of tomato plants, while PGPR at a concentration of 7.5 ml/L can maximize the number of leaves and roots of tomato plants; Aiman et al. [13] stated that the better pod length in French bean legumes was obtained after PGPR application one week after planting, whereas the application of PGPR in the vegetative phase was carried out once a week indicating that French bean seeds grew better.

One of the PGPR which is commonly used is *Bacillus subtilis*. The application of *Bacillus subtilis* has been used by several researchers on various types of plants, Wulansari et al. [14] and Mugiastuti et al. [15] on tomato plants, and Tuhuteru et al. [16] on red onion planted in sand beach land. The use of *Bacillus* type bacteria in this study is easy to obtain. Based on this, it is necessary to carry out an assessment of the growth and production of red onion through the application of *Bacillus subtilis* as a source of growth regulators.

2. Methods

The research was conducted at the Plant Disease Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture and at Experimental Farm, Faculty of Agriculture, Hasanuddin University, Makaassar City, South Sulawesi Province from February to July 2020.

The study used a split plot design. Each soil hole contains a spacing of 20 x 20 cm. The bulbs selected in the seed preparation activity were the ones that looked healthy or without wound or rot defects with a harvest age of 70, 75, and 80 DAS. The size of the land used was 1.2 x 2 m using plastic mulch, the distance between the plots was a ± 40 cm wide and used as drainage channel with the depth of drainage/height of the plot was ± 20 cm. Furthermore, the application of manure just before planting was done with each treatment dose of 2 kg / plot from a dose of 10 tons/ha or 0.04 kg/mulch hole.

Bacillus subtilis bacteria obtained from the Laboratory of Plant Diseases, Department of Plant Pests and Diseases, Hasanuddin University. The bulbs were soaked in *Bacillus subtilis* isolate before planting. The land is also applied with eco farming 5 days before planting. The planting is carried out with 1 tuber planting hole. Harvesting is done when the red onion plants are around 60 DAS, or 60% of the neck is soft, the plants fall, and the leaves turn yellow. Harvested red onion plants were then observed as the last data collecting and then weighing the bulb (after drying for 5 days).

Observation parameters taken included the rate of sprouting (days), number of leaves per plant (strands), number of tillers per plant, number of bulbs, weight of wet stover per plant (grams), weight of stover per plot (kg), weight of dry stover per plant (grams), dry stover weight per plot (grams), bulbs dry weight (grams), bulb diameter (mm), and bulb production (tonnes/ha). Data from these parameters were analyzed using t-sample analysis.

3. Results and Discussion

Application of *Bacillus subtilis* 10^8 (p2) resulted in the highest number of leaves, number of stems, number of bulbs, bulb diameter, and bulb wet weight among other treatments. While the highest sprouting rate was shown by control (p0). The effect of *Bacillus subtilis* on the number of leaves

IOP Conf. Series: Earth and Environmental Science **921** (2021) 012078 doi:10.1088/1755-1315/921/1/012078

(strands), sprouting rate (days), number of stems (tillers), number of bulbs, bulb diameter (mm), and bulb wet weight (grams) can be seen in Table 1.

Table 1. Interaction of *Bacillus subtilis* in growth parameters of red onion (*Allium ascalonicum*).

Parameter	PGR *Bacillus subtilis* (p)			
	Control (p0)	*Bacillus subtilis* 10^4 (p1)	*Bacillus subtilis* 10^8 (p2)	*Bacillus subtilis* 10^{12} (p3)
Number of Leaves (strands)	20,47[a]	21,17[a]	**22,43** [a]	21,13[a]
Sprouting Rate (days)	**4,30**[a]	3,70[a]	3,47[a]	3,67[a]
Number of Stems (tillers)	3,57[a]	4,03[a]	**7,27**[a]	6,07[a]
Number of Bulbs	3,73[a]	4,13[a]	**7,27**[a]	6,07[a]
Bulb Diameter (mm)	16,83[a]	19,89[a]	**27,41**[a]	19,61[a]
Bulb Wet Weight (grams)	53,07[a]	54,27[a]	**70,07**[a]	55,37[a]

Note: The numbers followed by the same letter (a, b, c) are not significantly different

Growth is a quantitative change in plants during the life cycle due to cell division and enlargement [13]. Growth is a process of increasing size, namely the volume, weight, and number of cells that are irreversible [17]. Plant growth is influenced by various genetic and environmental factors. Environmental factors trigger the emergence of genetic traits. Environmental factors include temperature, water availability, sunlight, the composition of the atmosphere, the composition of gases in the soil, pH, nutrients, and microbes [18].

There are two types of observations made in this study, namely growth observations (non-destructive) and results observations (destructive). Growth observations included the number of leaves, the number of stems (tillers), and sprouting rate. The results observation include bulb diameter, number of bulb, and bulb wet weight. Administration of *Bacillus subtilis* 10^8 (p2) resulted in the highest number of leaves, number of stems, number of bulbs, bulb diameter, and bulb wet weight among other treatments. Wulansari et al. [14] stated that *Bacillus* is able to increase plant growth through its functional characters, namely producing phytohormones and siderophores. A'yun et al. [11] revealed that phytohormones can increase the surface area of fine roots and increase the availability of nutrients in the soil. If the root growth is getting better, then the nutrients will be absorbed by the plants to support the photosynthesis process and the formation of plant cells that directly increase growth [19].

Application of *Bacillus subtilis* 10^8 (p2) showed the highest number of leaves compared to other treatments (Table 1). Leaves are one of the important organs in the photosynthesis process and determine plant growth and development as seen from the number of leaves [5]. The number of leaves affects the photosynthate that will be translocated into the bulbs, so that the more the number of leaves, the more photosynthate is produced from the photosynthesis process [10].

The value of the number of tillers directly proportional with the number of bulbs and bulb diameter (Table 1). The formation of tillers is a physiological process, in which a part of the bulb below the soil surface is morphologically transformed into an organ capable of storing large amounts of carbohydrates so as to encourage the formation of side tubers and tillers and leaves [20]. The high number of tillers is expected to produce a high number of bubls because each tiller can produce bulbs [8]. Bulb formation is influenced by the ability of plants to distribute photosynthate products to the leaves and bulbs [5]. Red onion bulbs can continue to grow and then form tillers when the minimum length of day is reached [7].

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This sprouting rate parameter is important to observe to find out which treatment gives the best results, so that it can shorten the time for red onion cultivation process if the sprouting speed of red onion bulbs is faster. Based on Table 1. It can be seen that the sprouting rate is not affected by the administration of *Bacillus subtilis*. However Hamid [20] states that PGR supports germination, namely stimulating auxins which play a role in stimulating the growth of meristems and cytokinins that play a role in spurring cell division and enlargement.

Application of *Bacillus subtilis* 10^{12} (p3) did not produce better growth than *Bacillus subtilis* 10^{8} (p2), possibly because the treatment of *Bacillus subtilis* 10^{8} (p2) was the optimum concentration to support the growth of red onion. Plants have the ability to regulate the optimum levels that are acceptable to support their physiological processes. As Tandi et al. [1] stated that the determination of the dosage for fertilization must be appropriate because it will not have an effect if it is not according to the needs of the plant. Elshyana et al. [4] stated that giving ZPT to plants is to encourage plant physiological processes, if the physiological processes in plants are already running, ZPT will no longer have a real effect.

4. Conclusion

The application of the *Bacillus subtilis* 10^{8} (p2) bacteria on red onion resulted in the highest number of leaves, number of stems, number of bulbs, bulb diameter, and bulb wet weight among other treatments. However, administration of *Bacillus subtilis* had no effect on the rate of sprouting.

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