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Research Article

Determination of the Effect of Salt Stress on Germination, Biochemical and Antioxidant Enzyme Activities in Linas Safflower Seeds

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Abstract: In this study, the germination and early seedling growth, biochemical and antioxidant enzyme activities (CAT, SOD, POD, and APX) of one-year, broad-leaved Linas safflower belonging to the Compositeae family were investigated at different salt concentrations (0, 50, 100, 150 and 200 mM). With increasing salt concentration, a 68.83% decrease in seedling length, 71% in stem length, 34% in germination rate, and 77% in fresh plant weight were determined. In addition, total phenolic content (267%), total flavonoid content (904%), CAT (462%), SOD (56%), POD (100%), and APX (381%) antioxidant enzyme activities were increased in parallel with the salt concentration. In addition, it was determined that as the salt stress increased, the water-soluble protein content decreased by 48%. In the study, it was determined that the seeds were relatively resistant to 100, 150, and 200 mM NaCl salt concentrations, and germination continued. As a result, it has been understood once again that our country has been feeling a negative impact lately, and the determination of alternative plants for growing oily plants has gained more importance in these days. Safflower, which is one of these plants, is a strategically important species both in terms of its oil content and being a source of biodiesel. This study carried out in this context will be a resource for our farmers regarding future studies on safflower seeds and which salt concentrations can be used for cultivation.

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1. Introduction

Most of the water resources in the world (70%) are salty. Therefore, considering that a quarter of the entire pedosphere (950 x 10^6 ha) is affected by salt, it shows up that 23% of the 1.5 x 10^9 ha cultivated land is saline (Glenn and O'Leary, 1985; Rhoades and Loveday, 1990; Flowers and Yeo, 1995). In addition, nearly half of the existing irrigation systems in the world (3 x 108 ha) are under the influence of secondary salinization, flooding, and alkalization, and therefore approximately 10 x 10^6 ha of irrigated land is abandoned each year (Szabolcs, 1987). Such unsuitable soils with low productivity are generally not suitable for agricultural production and cause yield loss. For these reasons, researchers have divided plants into halophytes and glycophytes. Halophytes are salinity tolerant plants that have adapted to saline environments and even benefit from high salt concentrations for optimum growth (Su

et al., 2020). On the other hand, glycophytes are salinity sensitive plants whose growth and development are adversely affected by soil salinity (Horie et al., 2012). Most cultivated plants are glycophytes. High salinity prevents the growth and development of glycophytes and severely limits productivity. Due to the increasing need for food production and the increasing distribution of salinity-affected soils, research on the response of plants to salinity has expanded rapidly in recent years. İncluding changes at the morphological, physiological, and molecular levels, studies of plant tolerance to salt stress cover many aspects of the effects of salinity on plant metabolism. In recent years, researchers have focused more on biotechnology, transgenic plants, improvement of breeding methodologies, and modification of the genetic structure of existing plants aimed at greater adaptation to salinity conditions. Physiological and biochemical changes caused by stress conditions have been frequently investigated by different researchers from past to present (Flowers et al., 1977; Greenway and Munns, 1980; Munns et al., 1983; Munns, 2002; Yildirim et al., 2021a; Yildirim et al., 2021b; Demir and Basayigit, 2021; Altun and Arslan, 2022).

Reactive oxygen species (ROS) synthesized under stress conditions increase free radicals in plant cells and cause oxidative stress in the cell. In such situations, plants develop antioxidant defense mechanisms to survive. Among these mechanisms, non-enzymatic antioxidants such as total phenolics, total flavonoids, and proline, and enzymatic antioxidants such as CAT, APX, SOD, and POD play an important role in the defense systems of plants against stress (Pérez-Pérez et al., 2012; Zhou et al., 2013; Shangguan et al., 2018).

Seed germination is the first and very important stage in the life cycle of plants. Germination is one of the most vital periods for a seed exposed to salinity (Dutta and Bera, 2014). Salinity prevents water absorption and seed germination by increasing the toxic effects of ions such as Na^+ and Cl^- (Turhan and Ayaz, 2004). Therefore, the salt tolerance of seeds during germination is critical for the growth of plants in saline soils (Khan et al., 1998). It is also known that high temperature interacts with salinity and increases the effect of stress conditions on the germination and development of many plants (Nedjimi, 2013). Therefore, the most basic approach to be followed in cases where the salinity problem in the soil can not be solved in the short term is to determine the salt tolerant species (Oral et al., 2019).

Safflower is an annual, broad-leaved, yellow, orange, red, white, and cream-colored oil plant from the family of Compositeae (Eryilmaz et al., 2014). In this study, it was purposed to determine the effect of salt stress on germination and antioxidant defense systems in Linas safflower seeds.

2. Material and Methods

In this study were used to Linas safflower cultivar seeds. The seeds were selected homogeneously, with uniform size, maturity, and appearance, and free from defects. The study was carried out on a total of 350 seeds, with 5 replications and 14 seeds in each replication. Plant seeds were first sterilized in 10% sodium hypochlorite for 10 minutes. Then, surface sterilization was performed with 80% ethanol and washed in distilled water for 3 x 5 minutes. Germination experiments of seeds were carried out in petri boxes containing double-layered sterile filter paper, 20/25ºC (in the dark) temperature, and blotter treated with different NaCl concentrations (0, 50, 100, 150, and 200 mM) for 7 days. Distilled water was used in the control treatment. Physiological parameters such as germination rate, seedling height, and stem length, and fresh weight were used to determine salt tolerance compared with control conditions. In addition, biochemical and antioxidant enzyme activities were also examined in plants during salt stress. Biochemical and antioxidant enzyme analyzes were carried out from the stem part of the seedling. For germination, the radicle length reached 10 mm (ISTA, 2019). For germination, the radicle was expected to reach a length of 10 mm (ISTA, 2019). The germinated seeds were counted every day to determine the germination percentage. Seedling length (cm), stem length (cm), germination rate (%), and fresh weight (mg/seedling) were determined at the end of the 7th day.

Total soluble protein content was determined according to the method of Hartree-Lowry (1972). Results were expressed as mg/ml

Total phenolic content was determined using the Folin-Ciocalteu method specified by Singleton and Rossi (1965). Results are expressed as mg GAE/g

Total flavonoid content was determined according to the method of Zhishen et al. (1999). Results are expressed as mg catechin/g.

POD activity was determined according to the method of Jiang et al. (2010). Results are expressed as Δ_{A460}/min/mg protein.

SOD activity was determined according to the method of Jiang et al. (2010). Results are expressed as U/mg protein.

APX activity was determined according to the method of Nakano vd. (1981). Results are expressed as mol/min/g protein.

CAT enzyme activity was determined according to the method of Beers vd. (1952). Results are expressed as U/mg protein.

The data obtained from the study were evaluated using the MINITAB package program. Tukey test was used to determine the differences between the means as a result of the evaluation.

3. Results and Discussion

In this study, the effect of salt stress on seedling length, stem length, germination, and fresh plant weight was found to be statistically significant ($p \leq 0.05$) (Table 1 and Figure 1). It was determined that there was a significant decrease in the seedling length as the salt concentration increased. The longest seedling length was obtained in the control treatment (13.83 cm), and the shortest seedling length was obtained in the treatment of 200 mM NaCl (4.31 cm). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 68% reduction in seedling length. Similarly, it was determined that the stem length decreased as the salt concentration increased. Accordingly, the longest stem length was obtained from the control treatment (13.33 cm) and the shortest stem length from the 200 mM NaCl treatment (3.80 cm). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 71% reduction in stem length. It is known that seed germination is inhibited in salty conditions, and they even lose their vitality in an environment containing high salt (Schmidhalter and Oertli, 1991; Tekin and Bozcuk, 1998). In our study, it was determined that there was a decrease in germination rates as the salt concentration increased. The highest germination rate was obtained from the control treatment at 92.5%, and the lowest germination rate was obtained from 200 mM NaCl treatment at 61%. When the control and 200 mM NaCl treatments were compared, it was determined that germination decreased by 34%. It was determined that the total fresh weight of the seedlings obtained as a result of seed germination decreased, similar to seedling length, stem length, and germination rate. While the total fresh weight of the seedlings obtained from the control treatment was 52.74 mg/seedling, it was determined as 12 mg/seedling in 200mm NaCl treatment. When the control and 200 mM NaCl treatments were compared, it was determined that there was a decrease of approximately 77% in fresh weight.

Table 1. The effect of salt applications on seedling height (cm), stem length (cm), germination percentage (%), and fresh plant weight (mg/seedling)

TREATMENTS	SEEDLING LENGTH (cm)	STEM LENGTH (cm)	GERMINATION RATE (%)	FRESH WEIGHT (mg/seedling)
Control	13.83±2.91^{a}	13.33±2.91^{a}	92.50±1.50^{a}	52.74±1.12^{a}
50 mM NaCl	12.70±3.16^{a}	12.01±3.15^{a}	82.50±2.50^{b}	42.69±2.21^{b}
100 mM NaCl	10.42±3.52^{b}	8.81±3.51^{b}	76.50±1.50^{c}	34.55±3,42^{c}
150 mM NaCl	8.13±1.77^{c}	6.83±1.77^{c}	70.00±1.85^{d}	19.26±2.23^{d}
200 mM NaCl	4.31±1.23^{d}	3.80±1.23^{d}	61.00±1.00^{e}	12.00±1.23^{e}

In this study, the effect of salt stress on total soluble protein, total phenolic, and total flavonoid content was found to be statistically significant ($p \leq 0.05$) (Table 2 and Figure 2). As a result of the excessive accumulation of ROS, protein degradation, DNA fragmentation, and cell death occur (Kusvuran et al., 2016). For this reason, it was determined that the amount of protein decreased as the salt concentration increased. The highest protein content was determined from the control treatment (2.65 mg/ml), and the lowest protein content was determined from the treatment of 200 mM NaCl (1.37 mg/ml). When the control and 200 mM NaCl treatments were compared, it was determined that there was a 48% decrease. Phenolic and flavonoid contents are at the turn of antioxidant defense mechanisms that are effective in tolerance to stress conditions in plants (Naczk and Shahidi, 2004). Because the accumulation of polyphenols in the cell is seen as a response to biotic and abiotic stress conditions. For

this reason, in this study, it was determined that there were increases in total phenolic and total flavonoid contents at different salt concentrations. The lowest total phenolic content was determined from the control group (3.07 mg GAE/g), while the highest was determined from the treatment of 200 mM NaCl (11.26 mg GAE/g). It was determined that there was a 267% increase between the control and 200 mM NaCl treatments. Similarly, it was determined that the total flavonoid content increased in parallel with the increase in salt concentration. The lowest total flavonoid content was determined from the control (0.25 mg catechin/g), and the highest was determined from the 200 mM NaCl (2.51 mg catechin/g) treatment. It was determined that there was a 904% increase between the control and 200 mM NaCl treatments.

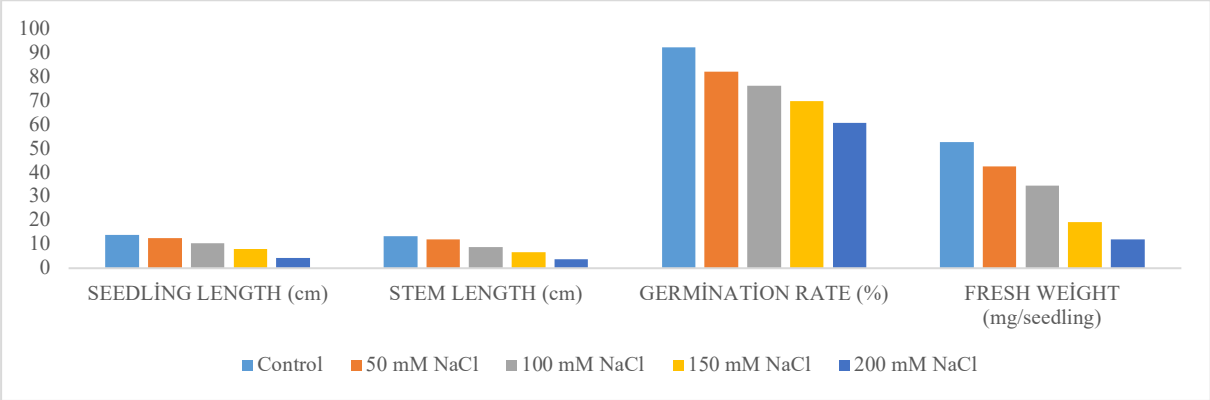

Figure 1. The effect of salt applications on seedling length (cm), stem length (cm), germination percentage (%), and fresh plant weight (mg/seedling).

Table 2. The effect of salt applications on total soluble protein (mg/ml), total phenolic content (mg GAE/g), and total flavonoid content (mg catechin/g)

TREATMENTS	TOTAL SOLUBLE PROTEIN (mg/ml)	TOTAL PHENOLIC CONTENT (mg GAE/g)	TOTAL FLAVONOID CONTENT (mg catechin/g)
Control	2.65±0.10^{a}	3.07±0.37^{d}	0.25±0.01^{c}
50 m MNaCl	2.21±015^{b}	4.88±0.37^{c}	0.36±0.01^{c}
100 mMNaCl	2.11±0.00^{b}	6.64±0.36^{b}	0.88±0.13^{b}
150 mMNaCl	1.69±0.21^{c}	7.24±0.16^{b}	1.15±0.13^{b}
200 mMNaCl	1.37±0.05^{c}	11.26±0.08^{a}	2.51±0.28^{a}

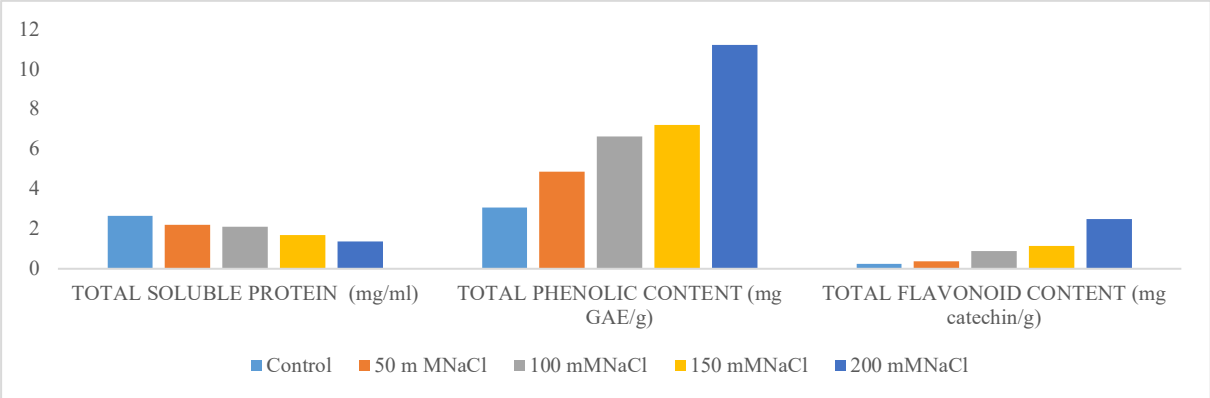

Figure 2. The effect of salt applications on total soluble protein (mg/ml), total phenolic content (mg GAE/g), and total flavonoid content (mg catechin/g).

In this study, the effect of salt stress on antioxidant enzyme activities was determined to be statistically significant ($p \leq 0.05$) (Table 3 and Figure 3). The highest activity in POD enzyme activity was determined from 150 mM NaCl (0.36 Δ_{460}/min/mg protein) treatment, while the lowest activity was

obtained from control and 50 mM NaCl treatments (respectively, 0.09 and 0.11 Δ A_{460}/min/mg protein). When the control and 150 mM NaCl treatments were compared, it was determined that there was a 300% increase. In SOD enzyme activity, it was determined that there was an increase in enzyme activity in parallel with salt concentration. The lowest SOD activity was similarly determined in the control treatment (6.29 U/mg protein) and the highest in the 150 mM NaCl treatment (11.13 U/mg protein). When the control and 150 mM NaCl treatments were compared, it was determined that there was an increase of approximately 77%. It was determined that there was an increase in the APX enzyme activity depending on the salt concentration, and the highest activity was obtained from the treatment of 200 mM NaCl (2.12 mol/min/g protein). When the control and 200 mM NaCl treatments were compared, it was determined that a 381% increase occurred. When plants are exposed to stress conditions, CAT antioxidant enzyme first contributes to the defense mechanism. It has been reported that the CAT enzyme clears high stability H_2O_2 and is the first antioxidant enzyme to respond at the onset of stress (Radi et al. 1991). In this study, the highest enzyme activity increase occurred in the CAT enzyme. The lowest enzyme activity was determined from the control treatment (8.07 U/mg protein) and the highest from the 200 mM NaCl (45.43 U/mg protein) treatment. When the control and 200 mM NaCl treatments were compared, it was determined that there was an increase of approximately 463%.

Table 3. The effect of salt applications on POD (Δ A_{460}/min/mg protein), SOD (U/mg protein), APX (mol/min/g protein), and CAT activity (U/mg protein)

TREATMENTS	SOD ACTIVITY (U/mg protein)	POD ACTIVITY (Δ A_{460}/min/mg protein)	APX ACTIVITY (mol/min/g protein)	CAT ACTIVITY (U/mg protein)
Control	6.29±0.64^{d}	0.09±0.00^{d}	0.44±0.08^{d}	8.07±1.76^{e}
50 mM NaCl	7.90±0.40^{c}	0.11±0.00^{d}	0.63±0.03^{d}	17.14±0.21^{d}
100 mM NaCl	9.02±0.51^{bc}	0.28±0.01^{b}	1.22±0.21^{c}	21.68±1.76^{c}
150 mM NaCl	11.13±0.55^{a}	0.36±0.01^{a}	1.71±0.16^{b}	32.74±1.68^{b}
200 mM NaCl	9.86±0.15^{ab}	0.18±0.02^{c}	2.12±0.16^{a}	45.43±0.95^{a}

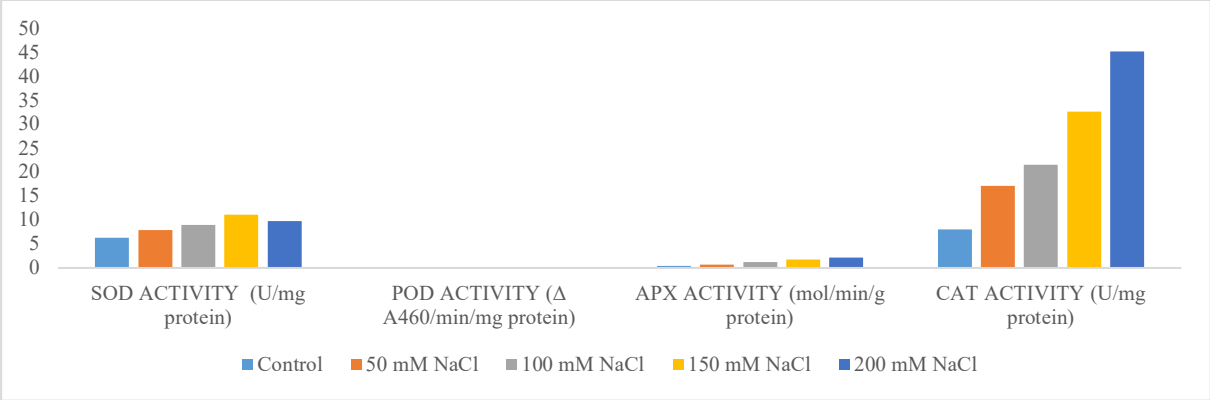

Figure 3. The effect of salt applications on SOD (U/mg protein), POD (Δ A_{460}/min/mg protein), APX (mol/min/g protein), and CAT activity (U/mg protein).

Salt stress occurs as a result of the excessive accumulation of soluble salts in the soil. The increase in salt content disrupts the ion and osmotic balance and suppresses plant growth and yield. (Parida and Das, 2005). Indeed, Yilmaz et al. (2022) reported that growth and development were adversely affected by the increase in salinity level. ROS accumulating in plant cells causes oxidative stress. (Jaleel et al., 2007). ROS accumulation is among the important factors that cause production and yield loss worldwide. ROS, oxidize proteins, damages nucleic acids, causes lipid peroxidation, and as a result, adversely affects many cellular functions (Foyer and Noctor, 2005). Indeed, Shah et al. (2021) reported that there was a significant decrease in germination percentage, seedling viability index, root and shoot lengths, and fresh and dry weights of seedlings under salt stress. Farhangi-Abriz and Torabian (2017) determined that different salt concentrations reduced shoot length and dry plant weight in bean seeds. Similarly, Paride and Das (2005) determined that seed germination and seedling growth were

negatively affected by salt stress. Huang et al. (2021) reported that salt stress decreased the germination percentage, emergence rate, and root and shoot length in sorghum seeds. As the salt concentration in the germination medium increases, the osmotic pressure increases, so the seed cannot receive the necessary water for germination (Day and Uzun, 2016). Therefore, it is known that plant germination is inhibited and germination percentage decreases in salty conditions (Bozcuk, 1989, Demir and Demir 1992; Dogan et al., 2008; Bahadorkhah and Kazemeini, 2014; Aydin and Atici, 2015; Kuscu et al., 2017; Toprak and Tuncturk, 2018; Kurtulus, 2020). In our study, it was determined that plant height, stem length, germination percentage, and plant fresh weight decreased as the salt concentration increased. Therefore, it was concluded that the obtained findings were compatible with previous studies and that salinity stress negatively affected plant growth (Abbasi et al., 2014, 2015; Liu et al., 2015). Plants develop some defense systems to resist the negative effects of ROS (Foyer et al., 1994). Antioxidant enzymes are the most important key elements of this system. ROS accumulation is neutralized by enzymatic antioxidant systems such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Mittler et al., 2004). SOD protects cells from damage by catalyzing oxygen radicals (O^{2-}) to hydrogen peroxide (H_2O_2). CAT, APX, and POD catalyze H_2O_2 into oxygen and water (Garratt et al., 2002). Shah et al. (2021) determined that there was an increase in total phenolic content, CAT, and SOD enzyme activities in parallel with the increasing salt concentration in corn seeds. It is reported that phenolic substances play an important role in scavenging free radicals, and their production increases under abiotic stresses (Król et al., 2014). In our study, it was determined that there was an increase in total phenolic and total flavonoid content with increasing salinity. Farhangi-Abriz and Torabian (2017) reported that different salt concentrations cause an increase in CAT, APX, SOD, and POD enzyme activities in bean seeds. Wang et al. (2021), determined an increase in SOD, POD, CAT and APX antioxidant enzyme activities in seedlings obtained from tomato seeds at different salt concentrations. Similarly, Altaf et al. (2021) reported that salt stress activates the antioxidant defense mechanism in plants and causes an increase in CAT, SOD, and APX enzyme activities. Many studies have been conducted in different plants regarding the antioxidant enzyme activities that take part in the plant defense mechanism under abiotic and biotic stress conditions, and as a result, it has been determined that there is an increase in enzyme activity (Bor et al., 2003; Li, 2009; Sharma et al., 2013; Turkhan et al., 2021; Yildirim et al., 2021a; Yildirim et al., 2021b; Boysan et al., 2022).

Conclusion

In this study carried out on Linas safflower seeds, 5 different NaCl concentrations (0, 50, 100, 150, and 200 mM) were applied, and the morphological, biochemical, and enzymatic responses of the seeds to salt stress conditions were investigated. In the study, it was determined that the seeds were relatively resistant to 100, 150, and 200 mM NaCl salt concentrations, and germination continued. In addition, it was determined that there were increases in biochemical and antioxidant enzyme results in parallel with salt concentration. It is thought that these increases in enzyme activity may be due to the defense mechanism developed to reduce the effect of stress. Due to the increasing world population, increasing salty soils, and decreasing freshwater resources, many studies have been carried out in recent years to identify new species that can adapt to adverse environmental conditions (Verma and Mishra, 2005; Ahmad et al., 2010; Maia et al., 2010; Zhang et al., 2013; García-Caparrós et al., 2019). In addition, it has been understood once again that our country has recently felt its impact negatively, and the determination of alternative plants for growing oily plants has gained more importance. Safflower, which is one of these plants, is a strategically important species both as an oil content and a source of biodiesel (Eryilmaz et al., 2014; Keskin, 2017). Therefore, more research is needed on this issue. This study carried out in this context will be a resource for our farmers regarding future studies on safflower seeds and which salt concentrations can be used for cultivation.

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