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Response of Yam (*Dioscorea alata*) to the Application of *Rhizophagus irregularis* and Potassium Silicate under Salinity Stress

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Abstract: Yam (*Dioscorea alata*) is a tropical plant that is considered critical for food security. The use of high fertilizer, low soil fertility, and insect pest infestation reduce yam tuber yield and quality. The present study was performed to determine the effect of potassium silicate and arbuscular mycorrhizal fungi (AMF) on yam cultivated under salinity stress. This study revealed that the combination of *Rhizophagus irregularis* (AMF) and Potassium silicate was more effective than their individual application on yam and were beneficial for overall analyzed characters. We observed the days to emergence and the average days to first leaf emergence decreased by 33.46% and 26.78%, respectively, the number of leaves increased by 45.23%, number of sprouts per seed tuber by 50%, vine length by 60.8%, vine length at harvest by 40.53%, the average leaf width by 53.79%, petiole length by 31.74%, tuber length by 43.84%, average diameter of tuber by 56.58%, and average number of tuber per vine by 46.15% in T5 treated plants. We also recorded that starch content increased by 21.89%, ascorbic acid by 61.51%, average moisture by 8.36%, TSS by 50%, and total sugar by 69.53% in T5 treated plants. The total phenol was found to be 1.53% higher in T3 applied plants, while the dry matter was 36.37% higher in T5 treatment. Furthermore, the enzymatic evaluation of MDA in leaves was found to be enhanced by 142% in T2. The enzyme 8-OHdG from the leaves sample was reported to be increased after T5 by 621.15%. Moreover, the amount of CAT was higher by 53.46% in T2 treated plants. Likewise, the amount of enzyme SOD and POX in leaves of *D. alata* enhanced by 30.91% and 51.15% T2 treatments respectively.

Keywords: arbuscular mycorrhizal fungi (AMF); yield parameters; yam; antioxidant enzyme activities

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1. Introduction

Yam (*Dioscorea alata* L.), alternatively known as Greater yam, is an important crop high in carbohydrates and primarily consumed in subtropical and tropical countries [1]. It belongs to the *Dioscoreaceae* family with a polyploid genome [2]. The tubers of yam possess considerable nutritional and medicinal properties, and the fresh roots of the yam are high in antioxidants and Vitamin C [3]. Several accessions have been maintained in germplasm collections, although more research into the chemical components of yam tubers is still required [4,5]. Salt stress is considered one among the severe constraints for crop production. The salts present in the soil may reduce the plant's ability to take-up water leading to reduced growth rate. Further, the excessive amount of salts in the transpiration stream of plants may damage the transpiring leaves cells causing further growth reductions [6].

Yam needs fertilizer doses for producing tuber successfully, but chemical fertilizers exhibit adverse effects on the soil microorganisms, as well as on human health. Therefore, to mitigate the hazardous effects of chemical fertilizers, we need to substitute them with biofertilizers. Indeed, biofertilizers can help mitigate these adverse effects by improving microbial populations and fertility of soil. They are non-toxic and eco-friendly because they are beneficial to both crop productivity and yield [7]. Still, its multiplication ratio is limited, with around 10–30% of the total harvested product used as seed materials [8]. Synthetic fertilizers have a negative impact on soil microbial community and human health [9].

The AMFs are usually referred to as bio-fertilizers. Their mutualistic relationship with plant roots is prevalent in the natural environment. AMF can be considered to develop several sustainable production technologies [10]. They offer additional essential plant inorganic nutrients to host plants and often improve plant growth and productivity in natural and stressed conditions. The AMF's functioning as a bio-fertilizer has the potential to enhance plants' ability to adapt to changing environments. Furthermore, potassium silicate is a source of both potassium (K) and highly soluble silicon (Si) that is utilized as a plant bio-stimulant. Silicon is one of the most helpful mineral elements in plant development and crop production that aid in promoting tolerance for salt and oxidative stresses.

Generally, plants use monosilicic acid as the primary source for acquiring Si, which exists as liquid in the soil [11]. Potassium is one of the plant's key nutrients, as it assists in the production of carbohydrates, protein synthesis, cell proliferation, development, and quality. Moreover, it has been proven to improve physiological processes, vegetative growth, osmoregulation, and antioxidant enzymatic activity, which is beneficial during salt stress [12,13]. As a result, Potassium silicate has the potential to enhance yields associated characteristics, seed production and quality, as well as nutrient (N, P, and K) uptake significantly [14]. Therefore, environmental-friendly management strategies involving the application of AMF and combinations with other components for increased crop productivity must be standardized.

AMF has also been acknowledged as a safe, cost-effective, and long-term strategy to boost plant resilience to a number of abiotic stressors. Moreover, the application of AMF enhances the organic matter in the soil by augmenting the plant's biological activity, nutrient uptake quality and microflora in the soil [15]. The application of silicon (Si) and AMF plays a crucial role in regulating plant growth and development under stress conditions, promoting stress tolerance by limiting detrimental ion intake, improving root hydraulic conductance and fluid uptake, and increasing water usage efficiency. Additional research is needed to exploit better the potential of yam as a staple crop for food security and its significant health impacts. The interaction of yams and AMF and its combination with other substances may increase the tuber yields and food value of *D. alata*, which is traditionally grown. In this study, we evaluated how salinity, potassium silicate, *R. irregularis*, and *R. irregularis* + potassium silicate affected key morphological and biochemical characteristics of *Dioscorea alata*.

2. Materials and Methods

2.1. Plant Material and Treatment Combination

The experiment was conducted in 2019–20 in Agriculture Research Farm Jharkhand, India, at latitude 24°22′18″ N, longitude 86°19′27″ E. The average daytime temperature observed was 26.5 °C (±6.0 °C), with 50–68% relative humidity. This experiment employed the Mini setts of Sree Karthika (*Dioscorea alata* L.) (CTRI, Thiruvananthapuram, Kerala, India), weighing around 25 g of the *Dioscorea alata* for planting directly in the pots. The crop was sown around mid-April and subsequently harvested in late January. The other package and practices followed are defined elsewhere [16].

2.2. *R. irregularis* and Potassium Silicate Application along with Salt Treatment

R. irregularis at a concentration of 100 spores/g was received from M/S Shri Ram Solvent Extractions Pvt. Ltd. Bangalore, (India). Before transplanting, AMF inoculation treatments included 100 g of infected propagules (mycelium, roots and spores). After this, the filtrate was made with 100 g of mycorrhiza inoculum in water (distilled) through a

15–20 m layered filter paper (Whatman, GE Healthcare, UK). The potassium silicate utilized in this investigation was analytically pure. In the case of potassium silicate treatments, 100 mL of 2.0 mM potassium silicate solution (Noble Alchem, Indore, India) was administered to each pot at a 15-day interval until crop maturity. There were 3 replicates of treatment and each treatment consisted of 15 pots that were distributed in a randomized complete block configuration. The plants were treated with 250 mL of sodium chloride solution at a concentration of 200 mM once a week for six weeks after sprouting to screen for salinity tolerance [17]. A list of treatments used in the present study is presented in Table 1.

Table 1. Details of treatment conditions performed in the present study.

Treatment Code	Composition
T1	Control (Treatment free)
T2	Salinity Stress
T3	Potassium silicate
T4	*R. irregularis*
T5	*R. irregularis* + potassium silicate

2.3. Observation of Morphological Characters

The yam plants were investigated with the given treatments for several morphological parameters. The data for days to emergence, and average days to first leaf emergence were determined at the initial stage of every plant. Further, the number of leaves and number of sprouts per seed tuber were calculated after the emergence of plants; vine length (after 40 days), average internodes length and vine length in cm at harvest were recorded. The average leaf width, petiole length at the time of maturity, tuber length, and average diameter of tuber were calculated in centimeters. Moreover, the average number of tubers per vine, weight of tuber in kg, tuber yield per vine kg, and stem girth in cm were observed after plant harvesting.

2.4. Observation of Chemical Analysis and Enzymatic Activity

In the present study, biochemical traits, i.e., Starch content (g/100 gm), ascorbic acid content (mg/100 gm), average moisture (%), TSS (°Brix), total sugars (g/100 gm), total phenols (mg/100 gm), and the dry matter (%), were recorded. The starch content was estimated with glucose, acid hydrolysis (ice-cold) with sulfuric acid, perchloric acid, and ethanol using absorption of 630 nm as reference point. The ascorbic acid content was determined with 2, 6- dichloro phenol indophenols dye, total soluble solids (TSS) were estimated using the drops of extract on a hand-held refractometer (values were expressed in °Brix), the total sugars were estimated by taking 0.5 g of dried and powdered material in 20–25 mg ethanol, and heating in a water bath for 2 hrs, and the total phenols were determined using the Folin–Ciocalteu method [7].

Further, the concentrations of Malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), Catalase (CAT), Superoxide dismutase (SOD), and Peroxidase (POX) were determined from the leaves of *Dioscorea alata* plants. Therefore, 0.5 g of fresh leaves was ground with sterilized quartz sand and 2.5 mL of PBS in a mortar. After this, homogenates of tissues were centrifuged, and the supernatant obtained was kept at −80 °C. Further, MDA and 8-OHdG assays were carried out using OxiSelect™ TBARS Assay Kit (Cell Biolabs, San Diego, CA, USA) and DNA Damage Competitive ELISA Kit (Invitrogen, Carlsbad, CA, USA). The activities of CAT, SOD, and POX were assessed using Amplex® Red Catalase Assay Kit (Invitrogen), Superoxide Dismutase Assay Kit (Sigma-Aldrich, Saint Louis, MT, USA), and Amplex® Red Peroxidase Assay Kit (Invitrogen) [18].

2.5. Statistical Analysis

Data analysis using the Statgraphics Centurion XVIII software package (version 18, StatPoint Technologies, Warrenton, VA, USA) was carried out. The differences between the means of treatment groups were based on the Newman–Keuls multiple-range test, whereas Pearson's correlation coefficient analysis was also performed using the Statgraphics Centurion XVIII software package.

3. Result

3.1. Morphological and Yield Response of *Dioscorea alata*

This study recorded the effect of potassium silicate, *R. irregularis*, and *R. irregularis* + potassium silicate combination treatments on *D. alata* plants cultivated under salinity stress. The merged treatment of T5 has been observed for its desired influence for the below discussed parameters, followed by T4, and T3. In contrast, T2 was observed to negatively impact overall analyzed morphological characters (Table 2). The minimum number of days to emergence and the average days to first leaf emergence were observed in T5 treated plants with a decrease of 33.46% and 26.78%, respectively, compared to T1. In contrast, maximum days to emergence and average days to first leaf emergence were recorded in T2 with corresponding increase of 67.12% and 45.52%. Further, significant increments in the number of leaves (45.23%), number of sprouts per seed tuber (50%) and vine length (60.8%) were recorded in T5 treated plants. However, the average internodes length was recorded minimum under T2 with a decrease of 43.96% and maximum under T3 with increase of 28.02%.

Table 2. Observations for morphological/yield variables under Control (T1), Salinity stress (T2), potassium silicate (T3), *R. irregularis* (T4) and *R. irregularis* + potassium silicate (T5) in *Dioscorea alata*.

Variables	Control (T1)	Salinity Stress (T2)	Potassium Silicate (T3)	*R. irregularis* (T4)	*R. irregularis* + Potassium Silicate (T5)
Days to emergence	22.11 ± 0.62b	36.95 ± 1.60a	18.85 ± 1.61c	17.22 ± 0.56d	14.71 ± 0.41e
Average days to first leaf emergence	24.23 ± 0.25b	35.26 ± 3.90a	22.08 ± 1.61b	20.54 ± 1.36bc	17.74 ± 1.66c
Number of leaves	60.86 ± 11.70c	61.36 ± 3.50c	71.97 ± 10.16bc	82.17 ± 6.36ab	88.39 ± 3.86a
Number of sprouts per seed tuber	1.38 ± 0.21b	0.79 ± 0.07c	1.44 ± 0.21b	1.69 ± 0.18b	2.07 ± 0.18a
Vine length (cm)	97.32 ± 17.84cd	66.33 ± 2.44d	120.81 ± 9.02bc	135.31 ± 35.89ab	156.50 ± 12.05a
Average internodes length (cm)	10.35 ± 1.22b	5.80 ± 0.51c	13.25 ± 0.50a	12.27 ± 0.88ab	13.09 ± 1.98a
Vine length at harvest (cm)	244.00 ± 6.65b	148.11 ± 12.31c	307.32 ± 17.67a	317.36 ± 13.45a	342.90 ± 40.25a
Average leaf width (cm)	7.12 ± 0.87b	4.65 ± 0.21c	8.58 ± 0.22b	8.66 ± 0.97b	10.95 ± 2.03a
Petiole length (cm)	8.16 ± 0.86b	4.82 ± 0.20c	8.02 ± 0.33b	9.43 ± 1.66ab	10.75 ± 1.51a
Tuber length (cm)	22.81 ± 1.77c	13.34 ± 0.28d	24.38 ± 1.74bc	25.36 ± 1.16b	32.81 ± 1.89a
Average diameter of tuber (cm)	5.62 ± 0.63c	2.89 ± 0.87d	6.54 ± 0.32bc	7.69 ± 1.30ab	8.80 ± 1.04a
Average number of tubers per vine	1.17 ± 0.12abc	0.64 ± 0.07c	1.05 ± 0.09bc	1.55 ± 0.49ab	1.71 ± 0.53a
Weight of tuber (kg)	0.78 ± 0.06bc	0.47 ± 0.05c	1.07 ± 0.06bc	1.48 ± 0.75ab	2.13 ± 0.72a
Stem girth (cm)	3.33 ± 0.43c	2.01 ± 0.14d	3.73 ± 0.12bc	3.94 ± 0.30b	5.01 ± 0.20a
Tuber yield per vine (kg)	1.03 ± 0.20bc	0.63 ± 0.10c	1.36 ± 0.08bc	1.84 ± 0.78ab	2.36 ± 0.57a

Means with the same letter are not significantly different at $p < 0.05$ based on the Student–Newman–Keuls test.

Moreover, the application of T5 was reported best, while T2 was having negative impact on other traits as well, i.e., the highest increase in vine length at harvest (40.53%), average leaf width (53.79%), petiole length (31.74%), tuber length (43.84%), average diameter of tuber (56.58%), and average number of tubers per vine (46.15%) were reported in T5 treated plants. In comparison, the decrease in vine length at harvest (39.29%), average leaf width (34.69%), petiole length (40.93%), tuber length (41.51%), average diameter of tuber (48.57%), and average number of tubers per vine (45.29%) were calculated in T2 given yam plants. Similarly, the maximum increments in weight of tubers (173.07%), stem girth (50.45%), and tuber yield per vine (129.12%) were recorded in T5 while the corresponding decrements in weight of tubers (39.74%), stem girth (39.64%), and tuber yield per vine (38.83%) in T2. Most of the morphological parameters were reported to have a similar response in terms of highest and lowest values under the application of T5 and T2, respectively (Table 2).

3.2. Biochemical Component/Enzymatic Evaluation

In the present study, we found that the combination in T5 was recorded with the best positive impact. In contrast, the treatment T2 was reported to reduce the values for overall analyzed characters among all the performed treatments in *D. alata*. It was observed that the starch content was significantly reduced in T2, by 39.29%, and increased in T5, by 21.89%. For ascorbic acid, T2 reported a decrease of 34.93%, while T5 reported an increase of 61.51%. Further, average moisture was 19.33% lower in T2 and 8.36% higher under the influence of T5. In the case of TSS, it was found that T2 reduced the concentration by 45.71%, while T5 increased the concentration by 50%. Similar results were obtained for total sugar where we observed that application of T2 produced a decrease of 43.99%, while application of T5 enhanced the level by 69.53%.

The total phenol was found to be minimum T2 (35.94% less), and maximum was recorded in T3 (1.53% higher) applied plants. The dry matter was recorded minimum in T2 (17.45%) given plants, and a maximum (36.37%) was found in T5 treatment. Furthermore, the enzymatic evaluation of MDA in leaves was found to be diminished by 6.15% in T5 and enhanced by 142% in T2. The enzyme 8-OHdG from the leaves sample was reported to be decreased after T3 by 9.61% and increased after T5 by 621.15%. Moreover, the amount of CAT was lower by 55.67% in T5 and higher by 53.46% in T2 treated plants. Likewise, the amount of enzyme SOD and POX in leaves of *D. alata* reduced by 15.42% and 12.72% in T5 and enhanced by 30.91% and 51.15% T2 treatments, respectively (Table 3).

Table 3. Observations for biochemical variables under Control (T1) and treatment of Salinity stress (T2), potassium silicate (T3), *R. irregularis* (T4) and *R. irregularis* + potassium silicate (T5) in *Dioscorea alata*.

Characters	Control (T1)	Salinity Stress (T2)	Potassium Silicate (T3)	*R. irregularis* (T4)	*R. irregularis* + Potassium Silicate (T5)
Starch content (g/100 g)	46.49 ± 0.54c	28.22 ± 1.74d	51.08 ± 2.16bc	53.06 ± 4.58ab	56.67 ± 1.63a
Ascorbic acid (mg/100 g)	12.94 ± 0.37b	8.42 ± 0.03c	13.92 ± 0.06b	16.96 ± 5.05ab	20.90 ± 2.23a
Average moisture (%)	55.28 ± 0.54c	35.95 ± 0.41d	58.18 ± 2.22bc	60.85 ± 5.15ab	63.64 ± 2.44a
TSS (°Brix)	7.00 ± 0.40b	3.80 ± 0.71c	7.85 ± 0.09b	8.79 ± 1.67ab	10.50 ± 1.87a
Total sugar (g/100 g)	4.66 ± 0.78c	2.61 ± 0.46d	6.05 ± 0.67bc	7.26 ± 1.45ab	7.90 ± 0.88a
Total phenol (mg/100 g)	85.56 ± 2.15a	54.81 ± 0.09b	86.87 ± 2.70a	85.70 ± 1.80a	86.30 ± 0.59a
Dry matter (%)	27.85 ± 0.95b	17.45 ± 0.64c	33.33 ± 1.55a	34.96 ± 3.77a	36.37 ± 1.26a
MDA (nM/g fresh weight)	69.44 ± 32.06b	168.05 ± 20.55a	81.17 ± 5.62b	77.46 ± 4.41b	65.17 ± 15.06b
8-OHdG (µg/g fresh weight)	2.08 ± 0.29c	2.54 ± 0.19c	1.88 ± 0.36c	5.18 ± 1.49b	15.00 ± 1.24a
CAT (kU/g fresh weight)	12.70 ± 1.10b	19.49 ± 2.44a	10.43 ± 2.18b	19.36 ± 2.53a	5.63 ± 1.75c
SOD (% activity IR)	71.60 ± 2.37b	93.73 ± 1.62a	68.04 ± 0.30b	71.27 ± 0.85b	60.56 ± 0.80c
POX (U/g fresh weight)	165.26 ± 26.08b	249.79 ± 131.05a	155.42 ± 1.11b	145.63 ± 0.70b	144.24 ± 2.23b

Means with the same letter are not significantly different with $p < 0.05$ based on the Student–Newman–Keuls test.

4. Discussion

The climate change and biodiversity deprivation, agricultural practices in the 21st century will confront enormous hurdles in producing sufficient healthy food for the increasing world's populations. As a result, establishing ways to improve crop productivity and quality has become a significant global agricultural issue [19]. The AMF application as a bio-inoculant may be a viable approach that provides significant long-term benefits in soil fertility, plant nourishment and protection, and promising potential in sustainable agriculture [20]. AMFs are the most widespread in the soil in organic agricultural systems, offering crucial agrosystem advantages. Furthermore, Bijalwan et al. [21], evaluated the effect of salinity and mycorrhizal and silicon application on various biochemical and morphological characteristics of watermelon and found a combination of silicon and mycorrhiza was more effective than each of them alone [21].

This study examined the consequences of salinity, potassium silicate, *R. irregularis* and a combination of *R. irregularis* and potassium silicate in *Dioscorea alata* on important morphological and biochemical traits. The observations for a number of morphology characters of yam were analyzed under given treatments. A combination of *R. irregularis* and potassium silicate have a beneficial impact on overall analyzed parameters, followed by a single *R. irregularis* and potassium silicate. Basically, the AMF has a symbiotic association with plant roots, influencing the metabolic activities of the host plants. As a result, we found that minimum days to emergence and the average days to first leaf emergence were recorded in the mixed application of T5, which may be associated with AMF symbiotic nature and its impact on metabolic activities resulting in the beneficial output of yam plants. Likewise, the highest number of leaves and sprouts per seed tuber and vine length was calculated in the T5 treated plants, while the lowest values were recorded under T2. The vine's growth is initially based on the planted sets' food reserve, with a well-settled root structure that begins to absorb nutrition from the soil and grows faster [22]. Similarly, Kumar et al. [7], reported that the number of leaves, sprouts per tuber, and vine length significantly increased with the application of *R. irregularis* and Azotobacter [7].

Soil salinization is well known for impairing plant growth and development, agronomic traits and minerals uptake is a major environmental issues across the globe [21,23]. For several reasons, plants benefit from mycorrhizal connections, including developing systemic resistance and stimulating antioxidative systems [18]. Several studies have shown that mineral nutrients or microorganisms can help plants to cope with salt stress, and silicon helps to reduce salt stress by regulating antioxidant enzymes [21]. In this study, we found that the silicon application significantly increased the average internodes length and found to be decreased salinity stress. The average leaf width, petiole length, diameter of tuber, and number of tubers per vine were also found to be the highest in the treatment T5. At the same time, T2 was recorded to have a negative impact on the above-discussed parameters. Likewise, the cumulative effect of *R. irregularis* and potassium silicate was recorded to increase the tubers weight, stem girth and tuber yield per vine. This study also observed that starch content, ascorbic acid, average moisture, TSS, and total sugar were decreased in salinity stress, but increased in the mixed application of *R. irregularis* and potassium silicate.

Stress can affect plant growth, development, and production by inducing morphological, physiological, biochemical, and metabolic alterations through various processes. Jabeen and Ahmad, [24] also reported the increased levels of proline, malondialdehyde (MDA), catalase (CAT), and peroxidase (POX) under salt stress in safflower and sunflower seedlings [24]. In this study, total phenol and dry matter were also increased under the influence of T3 and T5, while decreased in T2. Further, the enzymatic assessment of MDA, 8-OHdG, CAT, SOD, and POX was conducted from the shoot of *Dioscorea alata* plants treated with T2, T3, T4, and T5. In the same direction, T5 decreased the MDA production, and its production was found to be increased in T2. Apart from this, the enzyme 8-OHdG increased in T5 and decreased in T3. Moreover, CAT, SOD, and POX production was higher in T2 and lowered in T5 treatments. These results are also confirmed by Yadav et al. [25], where an increased antioxidant enzyme activity has been observed with increased salinity stress, suggesting that AMF inoculated plants may possess higher stability of membrane [25]. AMF and potassium silicate inoculation in plants is reported to have increased enzymatic activity. Reactive oxygen species (ROS) are considered harmful for they may lead to lipid-peroxidation in cell membrane, demanding a more robust antioxidant system for neutralization [26]. In this scenario, increased ROS-scavenging enzyme activity, such as superoxide dismutase and catalases, helps lower the ROS-induced cellular damage. Further, the AMF and Si application has also been shown to increase the antioxidant enzymes activity in *Brassica juncea* plants (particularly SOD) [27]. Similarly, the application of AMF and Si in combination was best for enhanced antioxidant efficiency of melon in salinity stress [21] and it was suggested that mycorrhizal application and silicon application may help overcome the salinity in watermelons.

5. Conclusions

Overall, this research can be helpful in determining the ideal growing conditions for improved yam production when it is cultivated or subjected to salt stress. Furthermore, in the present investigation, under salinity stress, we found that a mixed application of *R. irregularis* and potassium silicate was best to overall screened morphological, biochemical, and antioxidant defense parameters, what characterized by improved plant growth and development, followed by AMF and silicon treatment alone, while salinity stress wa found to reduce the overall performance of plants. The application of AMF and silicon together might be investigated at the cellular and molecular to better understand their effect under salinity stress in yam.

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