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Morpho-Cultural Characterization, Physicochemical Activities, and Effect of Abiotic Factors on the Growth of *Rhizobium* sp. Isolated from Nodules of 18 Soybean (*Glycine max* (L.) Merr.) Varieties Farmed in Cameroon

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Abstract

Acidic pH, high salinities, and extreme temperatures limit agricultural production in tropical soils, particularly in Cameroon. Rhizobia can improve crop growth and productivity in these soils, thanks to their ability to adapt to stressful conditions. In this study, nodules of 18 varieties of soybeans (*Glycine max* (L.) Merr.) were used to isolate rhizobia. The isolates were characterized and screened for their resistance to pH (2 to 6.8), salinity (NaCl 2% to 12%, w/v), and temperature (15˚C to 45˚C). Then, the physicochemical activity (catalase, proteins, total antioxidant capacity (TAC), ferrous reducing antioxidant power (FRAP), and malondialdehyde (MDA) of the isolates was assessed using referenced methods. The results showed that out of 108 isolates obtained, 73 were *Rhizobium* sp., endowed with variable pH, salinity, and temperature adaptabilities. The highest acid resistance (pH 2 to 4), salinity (4% to 12%), and extreme temperature (40˚C to 45˚C) were recorded with isolates 1M, 2M, 7M, and 5M. Isolate 10G2 showed the highest protein content (135.33 ± 5.65 µg/mL), while the isolates 1, 2G, 5P', and 13M scored the highest catalase activity (0.07 ± 0.00 µmol/mL/g prot). All the isolates demonstrated antioxidant activity, with the highest FRAP recorded by isolate 7M (298.46 ± 0.00 µg

AAE/mL) and the highest TAC by isolate 4M1 (1335.93 ± 10.84 µg AAE/mL). They also presented the ability to inhibit oxidative stress through the inhibition of MDA production. The lowest MDA value (1.14 ± 0.05 µmol/L) was obtained with the isolate 11G3. Generally, the isolates with interesting adaptation to abiotic conditions and physicochemical activities were from soybean varieties TGX 2007-11 F, TGX 2001-12 F, TGX 1991-22 F, SC Sentinel, Panorama 3, Maksoy 2N, Panorama 237, and Panorama 2. These results highlight the potential of *Rhizobium* sp. isolated from soybean nodules in improving the crop productivity of tropical soils and suggest further characterization of these strains using genomic approaches.

Keywords

Soybean, Nodules, *Rhizobium* sp., Adaptation, Abiotic Stress, Physicochemical Activity

1. Introduction

Around one-third of tropical soils worldwide are acidic [1]. In Cameroon, over 80% of cultivated lands are acidic [2]. Acidic soils are characterized by ecosystems marked by the presence of limiting factors for most soil microorganisms [3]. Under these conditions, there is a great variability in the physico-chemical characteristics of soils as well as their microbial diversity. Humid tropical soils, considered favorable environments for productivity worldwide and particularly in Africa, can become unfavorable places for the survival of beneficial soil microorganisms due to soil acidity [4]. Besides acidity, some factors limited microorganism-plant interaction in tropical soils, including salinity and extreme temperatures [5]. These interacting abiotic factors reduce the growth and survival of beneficial soil microorganisms such as rhizobia [6]. Under such environmental conditions, the survival and persistence of rhizobia depend on their adaptive mechanisms to mitigate the adverse effects of abiotic stresses such as elevated temperatures, soil acidity, and salinity. These adaptations are essential for maintaining the population density and ensuring effective symbiotic interactions with leguminous plants [7]. Considering the *rhizobium*-legume symbiosis, the factors likely to hinder biological nitrogen fixation are not the absence of efficient rhizobia, but the ecological factors limiting their potential [8]. According to Manet *et al.* [9], various abiotic stresses, including survival in soils, competitiveness for nodulation, and symbiotic efficiency, can affect the dynamics of rhizobial populations and their functional roles in the rhizosphere. These abiotic factors cannot only interfere with nodulation but also influence nitrogen-fixing activity after symbiosis establishment [10]. Abd-Alla *et al.* [11] reported that nitrogenase, involved in nitrogen-fixing activity, is highly sensitive to variation in soil osmotic potential. Besides nitrogen fixation, abiotic stress also leads to the activation of defense systems and lipid peroxidation [12]. The reactive oxygen species (ROS) produced at high rates when plants are exposed to stress generate oxidative damage in plants that is reflected in their malondialdehyde (MDA) content, a by-product of lipid peroxidation that is one of the first nonspecific links in the overall stress response [13]. In the literature, it was highlighted that the total antioxidant capacity of plants is linked to the symbiotic nitrogen uptake activity in legume root nodules and depends on the efficiency of the *rhizobium*-legume symbiosis [14]. Antioxidants, catalase, osmolytes, stress proteins, and exopolysaccharides are produced by rhizobia to survive in harsh environments caused by abiotic stress [15]. Ben-Laouane *et al.* [16] mentioned that rhizobia can improve the plant's tolerance to stress by inducing physical and chemical changes. Zhang *et al.* [17] reported that rhizobia can improve plant tolerance to low temperatures by affecting nitrogen metabolism and uptake. Rhizobia also play an important role in the management of oxidative stress by plants through the improvement of an antioxidant defense system, which includes an enzymatic system [18] and a non-enzymatic system [19].

The study of stress tolerance of rhizobia aims to find viable solutions for sustainable agriculture in the context of climate change and crop losses. To this end, researchers are mainly focused on legumes, which are the natural hosts for rhizobia that convert atmospheric nitrogen into plant-available nitrogen, thereby enhancing the nitrogen-fixing capacity of plants [20]. Indeed, an effective *Rhizobium*-legume symbiosis can ensure high yields and good nitrogen balance in depleted soils [21]. This requires an efficient *Rhizobium* capable of surviving in adverse environmental conditions and endowed with physicochemical activity, including inhibition of oxidative stress. For this purpose, nodules from soybean (*Glycine max* L. (Merr.) were chosen in this study as that oilseed crop is among the most common legumes grown in Africa [22], for its multiple food and industrial functions [23]. Also, owing to its efficient symbiosis, each year approximately 16.4 Tg of atmospheric nitrogen, constituting about 77% of the total nitrogen amount, is fixed by legumes [24]. In Africa, few research projects have been conducted on *Rhizobium* endowed with resistance to abiotic conditions and physicochemical activities. In Senegal and Tunisia, the resistance of *Rhizobium* strains to high temperatures (40˚C to 45˚C) and salinity (NaCl > 2%) was reported by Cacciari et *al.* [25]. In Cameroon, Ngo Nkot *et al.* [26] demonstrated that *Rhizobium* strains isolated from bambara groundnut can grow at low pH but might encounter difficulties at a NaCl concentration of 4%. Although the stress tolerance of rhizobia is the subject of numerous studies worldwide, knowledge remains limited concerning indigenous strains associated with soybean in poor, acidic tropical soils exposed to multiple abiotic stresses, particularly in Central Africa. In particular, the ability of these local strains to adapt to combined stress conditions (acidity, salinity, temperature) has not yet been systematically characterized. This study fills this gap by isolating and evaluating, for the first time in Cameroon, *Rhizobium* spp. strains according to their tolerance to abiotic stresses and their antioxidant responses, in view of identifying robust candidates for the development of bio-inoculants adapted to specific agroecological conditions. To our knowledge, there are few or no studies on the characteristics and *Rhizobium* profile of soybeans cultivated in acidic Cameroonian soils, as well as their physicochemical activities. It is in this context that the present study was designed. The objectives were to evaluate the tolerance to abiotic factors (acidity, salinity, and temperatures) of *Rhizobium* sp. cultures isolated from soybean varieties grown on acidic soils in the forest zone of Cameroon, and identify the physicochemical activity (antioxidant activity, inhibition of lipid peroxidation) of these isolates for select high-potential *Rhizobium* sp. isolates that could be good biofertilizer candidates.

2. Materials and Methods

2.1. Nodule Collection

The sampling site selected for this study is located in an experimental station of the Institute of Agricultural Research for Development (IRAD) at Nkolbisson (3˚51'N, 11˚27'E; altitude 769 m) in the Centre region of Cameroon. Root nodules were collected during March 2022 after complete pod filling with different soybean varieties originated from 7 countries as indicated in **Table 1**, and grown on an acidic soil (pH 4.10). Plant roots were carefully removed individually, wrapped in newspaper, and transferred to the laboratory, where detached nodules were stored at 4˚C. Root nodules were evaluated based on morphological characteristics, including shape, color, spatial arrangement on the roots, size, and microscopic features. Nodules exhibiting traits typically associated with active nitrogen fixation, such as a pink or reddish interior and a healthy, uniform structure, were selected for rhizobia isolation. Nodule size was classified according to the criteria of King and Purcell [27], where small nodules have a size ≤2.0 mm, medium with a size between 2.0 and 4.0 mm, and large with a size >4.0 mm.

2.2. Isolation of Rhizobia from Root Nodules

Rhizobia were isolated from root nodules following the method of Vincent [28] with some modifications. In the laboratory protocol, six healthy nodules showing a pink coloration due to the presence of leghemoglobin were collected from several soybean plants. The nodules were thoroughly washed with tap water to remove adhered soil particles and then separated from the roots. Healthy, intact root nodules were subjected to surface sterilization by immersion in a 3% calcium hypochlorite solution for 3 min, followed by three rinses with sterile distilled water. To confirm surface sterility, an aliquot of the final rinse water was plated on nutrient agar and incubated at 28 ˚C for 48 h. The absence of microbial growth indicated successful surface sterilization. After surface sterilization, the nodules were individually ground under aseptic conditions and streaked onto sterile Mannitol Yeast Extract Agar (YEMA) supplemented with Congo red, poured into Petri dishes. The Petri dishes were incubated for 7 days at 28˚C (Bioevopeak, China). After the incubation period, well-isolated colonies were picked up and purified by 3 successive streaking on a new sterile YEMA agar. Pure isolates were stored at −80˚C in sterile YEM broth containing 60% glycerol.

Table 1. Morphological and cultural characteristics of *Rhizobium* sp. isolates obtained from nodules of 18 soybean varieties grown on acidic soil of Cameroon.

Soybean variety names	Origin	Isolate growth rate (days)	Isolate diameter (µm)	Isolate structure	Mucus type	Appearance of isolates	Isolate shape	Varieties coding
TGX-1988-18F	DARS (MALAWI)	4	4	Thick	Viscous	Milky and sticky	irregular	14
S1180/5/54 (N)	SeedCo (ZIMBABWE)	3	4	Thick	Viscous	Whitish and sticky	roundness	15
TGX 2001-10 DM	IITA (ZAMBIA)	3	2	Thick	Viscous	Whitish and sticky	roundness	16
SC Sentinel	SeedCo (ZIMBABWE)	3	2	Thick	Viscous	Whitish and sticky	roundness	5
Songda	Savanna Agricultural Research Institute of Ghana (CSIR_SARI)	3	2	Thick	Viscous	Milky and sticky	roundness	8
TGX 2007-11 F	IITA (CAMEROON)	4	4	Thick	Viscous	Whitish and sticky	roundness	1
TGX 2001-12 F	IITA (CAMEROON)	3	2	Thick	Viscous	Milky	irregular	2
PANORAMA 1	Semillas Panorama SAS (Colombia)	3	2	Thick	Viscous	Milky	irregular	6
TGX 1991-22 F	DARS (MALAWI)	3	< 2	Thick	Very viscous	Milky	roundness	4
SC SIGNAL	SeedCo (ZIMBABWE)	3	< 2	Thick	Very viscous	Milky	roundness	9
MAKSOY 1N	U.Makerere (UGANDA)	3	< 2	Thick	Very viscous	Milky	roundness	18
PANORAMA 237	Semillas Panorama SAS (Colombia)	4	4	Thick	Viscous	Whitish	irregular	11
TGX-1989-60 F	DARS (MALAWI)	4	2	Thick	Viscous	Whitish	irregular	12
MAKSOY 2N	U.Makerere (UGANDA)	4	4	Thick	Viscous	Whitish	roundness	10
TGX 2010-3 F	IITA (CAMEROON)	4	4	Thick	Viscous	Whitish	irregular	3
PANORAMA 2	Semillas Panorama SAS (Colombia)	3	4	Thick	Viscous	Milky	roundness	13
PANORAMA 3	Semillas Panorama SAS (Colombia)	3	2	Thick	Very viscous	Milky	roundness	7
TGX 1835-10 E	IITA (CAMEROON)	3	4	Slightly thick	Viscous	Whitish	roundness	17

2.3. Cultural Characterization of Isolates

2.3.1. Morphology of Isolates

From frozen stock, each pure isolate was streaked on sterile YEMA followed by incubation at 28˚C for 7 days. After incubation, the morphology characteristics of the isolates (diameter, texture, color, appearance, and shape) were assessed.

2.3.2. Adaptation Tests under Stress Conditions

The tolerance of *Rhizobium* sp. isolates to various abiotic factors such as pH, temperature, and salinity was assessed. Morpho-cultural characteristics of the different isolates, including the growth speed and colonies' color on YEMA supplemented with red Congo, and the formation of mucus, were used as criteria for selecting isolates that were tested in the different treatments.

Regarding salinity tolerance, 4 mL of sterile YEM broth supplemented with NaCl at concentrations ranging from 1% to 12% (w/v) was inoculated with a pure colony of each *Rhizobium* isolate. Control cultures were prepared by inoculating the broth without NaCl. All cultures were incubated at 28˚C for 7 days under shaking conditions at 120 rpm. Seven days was chosen as the optimal growth period of the selected isolates from preliminary experiments. Bacterial growth was assessed by measuring the optical density at 620 nm against the blank made from uninoculated broth treated under the same conditions (UV-Mini 1240, Shimadzu, Japan).

Concerning temperature, tubes containing 4 mL of sterile YEM broth were inoculated with pure colonies of the rhizobia, and the tubes were incubated at different temperatures ranging from 15˚C to 45˚C for 7 days. The rhizobial load was measured by reading the optical density of tubes at 620 nm.

To assess the effect of pH on the growth of rhizobia, 4 mL of sterile YEM broth was introduced into sterile tubes, and the pH of the broths was adjusted from 2 to 6.8 using 1N hydrochloric acid (HCl) and 1N sodium hydroxide (NaOH). The pH-adjusted media were then buffered with Citrate buffer to maintain a stable pH during bacterial growth. The cultures were incubated at 28˚C, and growth was measured by optical density at 620 nm.

2.4. Tolerance of the Isolates to Abiotic Stress Conditions

2.4.1. pH Tolerance

The ability of each *Rhizobium* sp. isolate to grow under acidic conditions was determined by inoculating each isolate into tubes containing YEM broth with pH adjusted to 2.0, 2.5, 3, 3.5, 4, 4.5, 5, 6.8, followed by incubation for 7 days at 28˚C. The optical density of the tubes was read at 620 nm.

2.4.2. Salt Tolerance

The ability of the isolates to grow at different NaCl concentrations was assessed by inoculating each isolate into YEM broth containing 1%, 2%, 4%, 6%, 8%, 10%, and 12% NaCl. Cultures were incubated at 28 ˚C for 7 days under shaking conditions at 120 rpm to ensure adequate aeration. Bacterial growth was evaluated by measuring the optical density at 620 nm.

2.4.3. Temperature Tolerance

The ability of the isolates to grow at different incubation temperatures was evaluated by inoculating each of the 73 rhizobia isolates into YEM broth. The inoculated cultures were then incubated at 15˚C, 20˚C, 25˚C, 30˚C, 35˚C, 40˚C, and 45˚C for 7 days under shaking conditions at 120 rpm to ensure adequate aeration. Bacterial growth was assessed by measuring the optical density at 620 nm.

2.5. Physicochemical Activity of the Isolates

2.5.1. Extract Preparation

A well-isolated colony of each *Rhizobium* sp. was introduced into a conical flask containing 100 mL of sterile YEM broth. The inoculated tubes were incubated at 28˚C for 7 days. After incubation, the inoculated broths were centrifuged at 4000 rpm for 20 min (Rotofix 32A, Hettich, Germany), and the cell-free supernatants were collected and stored for testing.

2.5.2. Protein Content

The method of Lowry *et al.* [29] was used to assess the protein content of cell-free supernatants. Briefly, 1 mL of the supernatant was mixed with 5 mL of copper reactive solution (0.5 mL of $CuSO_4$ 1% (w/v), 0.5 mL of 2% sodium tartrate (w/v), and 50 mL of sodium carbonate 2% (w/v) in NaOH 0.1 M). After thoroughly mixing, the mixture was left at room temperature (25˚C ± 1˚C) for 10 min, and 0.5 mL of Folin-Ciocalteau was added. The mixture was incubated for 30 min at room temperature, and the absorbance was read at 750 nm against the blank. SAB at different concentrations was used as a standard to draw the calibration curve ($r^2 = 0.94$) that was used to calculate the protein content of the samples.

2.5.3. Antioxidant Activities

The antioxidant capacity of the cell free supernatants from the culture of *Rhizobium* sp. isolates in YEM broth at 28˚C for 7 days was assessed using 3 methods: the phosphomolybdenum method to evaluate total antioxidant capacity (TAC), the ferric reducing antioxidant power (FRAP) method to evaluate iron reduction power, and catalase.

Ferric Reducing Antioxidant Power (*FRAP*)

The method of Oyaizu [30] was used to assess the ferric-reducing power of *Rhizobium sp.* isolates. A volume of 1 mL of cell-free, was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferrocyanide (1%) and incubated in a water bath at 50˚C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was centrifuged at 650 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue-green color was measured at 700 nm. Ascorbic acid was used as a positive control.

Determination of total antioxidant capacity

The total antioxidant activity of the extract was assessed by the formation of a phosphomolybdenum complex [31]. To this end, 0.2 mL of cell-free supernatant was added to 2 mL reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Absorbance was measured at 765 nm after 60 min of boiling. Ascorbic acid was used as the standard, and total antioxidant capacity was expressed in milligrams of ascorbic acid equivalent (AAE) per gram of dry matter extract (mg AAE/gDM).

Catalase activity

The method of Sinha [32] used in this study was based on the fact that catalase present in the cell-free supernatant reduces hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). The remaining H_2O_2 molecules are bound by potassium dichromate to form a blue-green precipitate of unstable perchloric acid. That precipitate is then decomposed by heat treatment to form a green complex absorbing at 620 nm. Catalase activity was proportional to optical density and was determined by the calibration curve ($r^2 = 0.94$). The activity was expressed in mmoles of H_2O_2 decomposed per mg of protein.

2.5.4. Inhibition of Lipid Peroxidation

To assess the ability of rhizobia to inhibit lipid peroxidation due to ROS generated by stress conditions, the method described by Wilbur *et al.* [33] with slight modifications was used. In the protocol, 100 µL of cell-free supernatant was mixed with 250 µL of trichloroacetic acid 20% (w/v) and 400 µL of thiobarbituric acid 0.67% (w/v). The mixture was transferred into glass tubes and the tubes were sealed, heated at 100˚C for 15 min, and cooled in a water bath for 30 min. Then, the tubes were opened to allow gas evaporation, centrifuged (1500 rpm, 5 min), and the optical densities were read at 532 nm against a solution of NaCl 0.9% used as a blank. MDA concentration was expressed in mmol/mL of supernatant.

2.6. Statistical Analysis

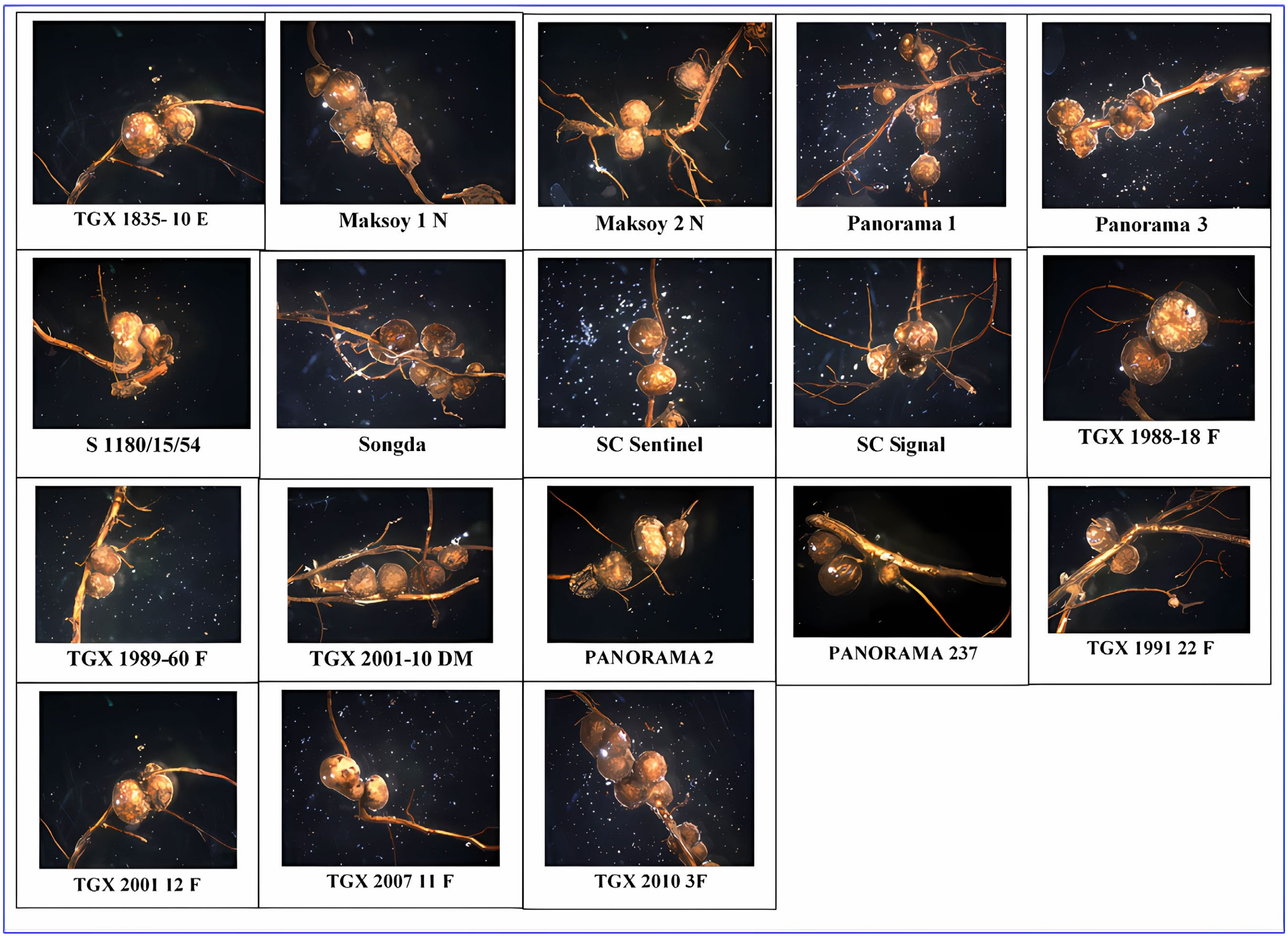
All experiments were repeated at least three times, and the data obtained are presented as means ± standard deviations. The experimental design followed a completely randomized design, with three biological replicates per treatment. Data were submitted to analysis of variance (ANOVA) using Statgraphics Centurion XV version 16.1.18 (StatPoint Technologies, Inc., Virginia, USA), and comparisons between means were performed using Duncan's multiple-range test. The significance level was set at 5%. Pearson correlation was used to assess the relationship between the 73 *Rhizobium* sp. isolates, abiotic factors, and biochemical tests. Principal component analysis (PCA) was performed to visualize the association between the different parameters analyzed with the 73 isolates using XLSTAT software version 2014.5.03 (Addinsoft, Inc., New York, USA).

3. Results

3.1. Nodule Characteristics

The nodules of the 18 soybean varieties were rounded or oval, as shown in **Figure**

1. These shapes varied from one soybean variety to another. They were large lobular shapes with a light-brown color (TGX 2007 11 F and Panorama 2 varieties) and globular clustered shapes with a brown color along the roots and small-sized absorbent hairs (Figure 1). These nodules were distributed in small numbers with lateral roots and small absorbent hairs. For *Rhizobium* sp. isolation, healthy, uninterrupted, and pink nodules were chosen, as these characteristics indicate their maturity and also that symbiosis had taken place.



Pictures were taken with an Olympus SZH10 SCOP PRO microscope, series 1S2219; magnification: 7X, width: 8.25 µm; pixel height: 8.25 µm; XY ratio: 1.00000; zoom 33%; image info: 5000 µm (Manet, 2022).

Figure 1. *Rhizobium* sp. root nodules of the 18 soybean varieties.

3.2. Characteristics of *Rhizobium* sp. Isolates

Nodulation tests were carried out on 108 *Rhizobium* sp. isolates from 18 soybean varieties, enabling 73 isolates to be selected for their ability to nodulate effectively. The remaining 35 isolates, although showing no nodulation ability, were kept in the lab for further studies. Gram staining revealed that all 73 isolates of *Rhizobium* sp. were Gram-negative. In all, the 73 *Rhizobium* sp. isolates obtained were studied for further experiments. They were fast-growing isolates, forming visible colonies on yeast extract mannitol agar (YEMA) within 72 to 96 h of culture (Table

1). The isolates ranged in diameter from 2 to 4 µm. They displayed a thick structure, with viscous mucus of a whitish, milky appearance. The colony morphology varied from round to irregular forms.

3.3. Effect of pH Variation on the growth of the 73 *Rhizobium* sp. Isolates

As shown in Table 2, many isolates showed their strongest growth at pH 5, with optical density values at 620 nm ranging from 0.90 ± 0.01 (isolate 6G2) to 1.57 ± 0.01 (isolate 3M'''), indicating a nearly 75% increase in growth between the lowest and highest performances at this pH. However, several isolates showed optimal growth at other pH levels, including pH 3 (16M, 16M3 and 17G1), pH 3.5 (7M, 15G, 15M3 and 16M3), pH 4 (7M, 12G2), pH 4.5 (isolate 1) and pH 6.8 (1G'', 1M, 6G2', 6G3, 7M', 14P3, 17G4 and 17M), where OD ranged from 0.78 to 1.52. This wide distribution of optimal pH conditions and the magnitude of growth differences, up to twofold between isolates depending on pH levels, suggests substantial physiological or ecological variation between strains, which may suggest specific adaptations to local soil conditions or host legume genotypes.

Table 2. Growth of *Rhizobium sp.* isolates at different pH levels in terms of optical density 620 nm.

Isolates	pH = 2	pH = 2.5	pH = 3	pH = 3.5	pH = 4	pH = 4.5	pH = 5	pH = 6.8
1	$0.11 \pm 0.00^{fghijklmnop}$	0.06 ± 0.00^{abcd}	0.16 ± 0.00^{abcdefgh}	0.32 ± 0.03^{cdef}	0.52 ± 0.03^{ijklmnop}	1.28 ± 0.02^{r}	0.58 ± 0.00^{jklmn}	0.58 ± 0.00^{mnopqrst}
1G	0.07 ± 0.00^{abcdefg}	0.07 ± 0.00^{abcd}	0.06 ± 0.00^{ab}	$0.64 \pm 0.00^{abcdefghijk}$	0.61 ± 0.00^{abcdef}	0.19 ± 0.01^{abcdefg}	0.67 ± 0.00^{mnopqrs}	0.55 ± 0.03^{d}
1G'	0.06 ± 0.00^{abcde}	0.07 ± 0.00^{abcde}	0.07 ± 0.00^{ab}	0.35 ± 0.00^{defgh}	$0.51 \pm 0.01^{hijklmnop}$	$0.26 \pm 0.02^{abcdefghijk}$	0.82 ± 0.00^{abcde}	0.84 ± 0.01^{abc}
1G''	$0.08 \pm 0.00^{abcdefghij}$	0.07 ± 0.02^{abcd}	0.17 ± 0.01^{bcdefgh}	0.41 ± 0.04^{efghijk}	0.57 ± 0.04^{mnopqr}	0.19 ± 0.03^{abcdefg}	0.82 ± 0.02^{abcde}	0.93 ± 0.02^{ab}
1M	0.07 ± 0.00^{abcdef}	0.06 ± 0.00^{abcd}	0.06 ± 0.00^{ab}	0.35 ± 0.00^{defghi}	0.31 ± 0.01^{bcd}	0.44 ± 0.02^{ijklmno}	0.17 ± 0.00^{bc}	1.00 ± 0.00^{a}
1P	$0.15 \pm 0.00^{abcdefghi}$	0.06 ± 0.00^{abcd}	0.59 ± 0.00^{abcdef}	0.71 ± 0.00^{abcde}	0.76 ± 0.02^{abcd}	0.17 ± 0.04^{abcde}	0.95 ± 0.01^{abcd}	0.83 ± 0.03^{abcd}
2	$0.10 \pm 0.00^{bcdefghijkl}$	0.40 ± 0.1^{a}	0.51 ± 0.05^{abcdefg}	0.31 ± 0.12^{cdef}	0.65 ± 0.01^{abcdef}	0.46 ± 0.06^{jklmno}	0.83 ± 0.02^{abcde}	0.74 ± 0.00^{opqrstuv}
2G	0.18 ± 0.00^{abcdef}	0.06 ± 0.00^{ab}	0.07 ± 0.01^{abc}	0.74 ± 0.00^{abcde}	0.57 ± 0.13^{mnopqr}	0.14 ± 0.03^{abcd}	1.15 ± 0.00^{abc}	0.75 ± 0.01^{pqrstuvw}
2M	$0.11 \pm 0.01^{fghijklmnop}$	0.06 ± 0.00^{ab}	0.21 ± 0.05^{efghi}	$0.61 \pm 0.01^{abcdefghij}$	$0.49 \pm 0.07^{ghijklmno}$	$0.32 \pm 0.12^{bcdefghijkl}$	1.01 ± 0.00^{ab}	0.68 ± 0.02^{jkl}
2M'	0.18 ± 0.00^{abc}	0.07 ± 0.01^{ab}	0.21 ± 0.12^{gh}	0.74 ± 0.23^{abcd}	0.67 ± 0.16^{abcde}	0.71 ± 0.63^{pq}	1.11 ± 0.28^{abcd}	0.80 ± 0.05^{abcdef}
2P'C'	$0.08 \pm 0.00^{abcdefghijk}$	0.07 ± 0.00^{abcde}	0.37 ± 0.06^{jklm}	$0.55 \pm 0.02^{mnopqrstu}$	0.70 ± 0.07^{abcdef}	0.77 ± 0.15^{q}	1.21 ± 0.00^{ab}	0.77 ± 0.00^{abcdef}

Continued

3G	0.12 ± 0.01^{fghijklmnopq}	0.08 ± 0.00^{abcdefg}	0.33 ± 0.07^{ijkl}	0.65 ± 0.01^{abcdefghij}	0.83 ± 0.00^{ab}	0.38 ± 0.00^{defghijklm}	0.86 ± 0.02^{abcde}	0.73 ± 0.00^{mnopqrstu}
3M'	0.11 ± 0.00^{efghijklmn}	0.16 ± 0.00^{ijk}	0.43 ± 0.03^{lmnopqr}	0.69 ± 0.01^{abcdef}	0.51 ± 0.00^{hijklmnop}	0.18 ± 0.04^{abcdefghi}	0.64 ± 0.00^{lmnopqr}	0.72 ± 0.00^{lmnopqrs}
3M'''	0.08 ± 0.00^{abcdefghi}	0.29 ± 0.00^{mn}	0.53 ± 0.05^{abcdefg}	0.48 ± 0.19^{jklmnop}	0.52 ± 0.00^{ijklmnop}	0.21 ± 0.00^{abcdefghi}	1.57 ± 0.01^{a}	0.60 ± 0.01^{ef}
4M1	0.18 ± 0.00^{abcdef}	0.06 ± 0.00^{ab}	0.45 ± 0.00^{mnopqrs}	0.39 ± 0.03^{efghij}	0.57 ± 0.04^{mnopqr}	0.14 ± 0.05^{abcd}	0.86 ± 0.00^{abcde}	0.71 ± 0.00^{klmnopq}
4M3	0.08 ± 0.00^{abcdefgh}	0.05 ± 0.00^{a}	0.37 ± 0.00^{jklm}	0.54 ± 0.07^{mnopqrst}	0.84 ± 0.04^{ab}	0.76 ± 0.08^{q}	0.55 ± 0.10^{ijkl}	0.77 ± 0.00^{abcdef}
5G	0.13 ± 0.01^{klmnopqrs}	0.06 ± 0.00^{ab}	0.19 ± 0.01^{cdefgh}	0.26 ± 0.00^{bcd}	0.72 ± 0.01^{abcde}	0.22 ± 0.00^{abcdefghi}	1.25 ± 0.00^{a}	0.76 ± 0.02^{rstuvw}
5M	0.12 ± 0.01^{jklmnopqrs}	0.05 ± 0.00^{a}	0.64 ± 0.03^{abcde}	0.45 ± 0.04^{ghijklm}	0.78 ± 0.02^{abc}	0.47 ± 0.18^{klmno}	1.20 ± 0.01^{ab}	0.76 ± 0.00^{stuvw}
5M''	0.22 ± 0.00^{abcde}	0.06 ± 0.00^{ab}	0.75 ± 0.05^{abcd}	0.71 ± 0.00^{abcde}	0.72 ± 0.00^{abcde}	0.42 ± 0.01^{ghijklmn}	1.00 ± 0.00^{abc}	0.86 ± 0.00^{ab}
5M2'	0.07 ± 0.00^{abcdefg}	0.06 ± 0.00^{ab}	0.05 ± 0.00^{a}	0.34 ± 0.03^{defg}	0.32 ± 0.03^{cde}	0.25 ± 0.00^{abcdefghijk}	1.03 ± 0.00^{abc}	0.66 ± 0.01^{hij}
5P	0.06 ± 0.00^{abc}	0.07 ± 0.00^{abcd}	0.06 ± 0.00^{ab}	0.46 ± 0.00^{hijklm}	0.37 ± 0.06^{def}	0.61 ± 0.07^{mnopq}	0.59 ± 0.00^{klmno}	0.89 ± 0.03^{ab}
5P'	0.06 ± 0.00^{abcde}	0.08 ± 0.00^{abcdefg}	0.08 ± 0.00^{abcd}	0.22 ± 0.00^{bc}	0.77 ± 0.02^{abc}	0.65 ± 0.01^{nopq}	1.05 ± 0.02^{abcd}	0.81 ± 0.00^{abcdefg}
6G	0.10 ± 0.00^{defghijklmn}	0.06 ± 0.00^{abcd}	0.19 ± 0.18^{cdefgh}	0.59 ± 0.00^{abcdefghij}	0.55 ± 0.01^{lmnopq}	0.26 ± 0.00^{abcdefghijk}	1.13 ± 0.00^{abcd}	0.76 ± 0.06^{rstuvw}
6G'	0.09 ± 0.00^{bcdefghijkl}	0.06 ± 0.00^{abc}	0.47 ± 0.00^{mnopqrst}	0.76 ± 0.00^{abc}	0.75 ± 0.03^{abcde}	0.13 ± 0.01^{ab}	1.04 ± 0.02^{abcd}	0.77 ± 0.01^{abcdef}
6G2	0.22 ± 0.02^{abcde}	0.09 ± 0.01^{abcdefg}	0.54 ± 0.03^{abcdefg}	0.80 ± 0.00^{ab}	0.53 ± 0.14^{jklmnop}	0.15 ± 0.00^{abcd}	0.90 ± 0.01^{abcd}	0.72 ± 0.02^{mnopqrs}
6G2'	0.14 ± 0.01^{abcdefghi}	0.08 ± 0.02^{abcdef}	0.10 ± 0.01^{abcdef}	0.56 ± 0.02^{mnopqrstuv}	0.67 ± 0.01^{abcdefg}	0.16 ± 0.02^{abcde}	0.81 ± 0.00^{abcde}	1.00 ± 0.00^{a}
6G3	0.10 ± 0.00^{cdefghijklm}	0.05 ± 0.01^{a}	0.39 ± 0.01^{klmn}	0.39 ± 0.00^{efghij}	0.58 ± 0.00^{opqrs}	0.37 ± 0.01^{cdefghijklm}	0.68 ± 0.00^{mnopqrst}	0.93 ± 0.01^{abc}
6G4	0.12 ± 0.00^{ijklmnopqrs}	0.06 ± 0.00^{abc}	0.40 ± 0.00^{lmnop}	0.59 ± 0.00^{abcdefghij}	0.57 ± 0.04^{mnopqr}	0.26 ± 0.22^{abcdefghijk}	0.87 ± 0.00^{abcde}	0.68 ± 0.01^{ijkl}
7M	0.19 ± 0.00^{abcdef}	0.19 ± 0.04^{jkl}	0.50 ± 0.01^{abcdefgh}	1.07 ± 0.04^{ab}	1.38 ± 0.08^{a}	0.20 ± 0.04^{abcdefgh}	0.86 ± 0.03^{abcde}	0.67 ± 0.00^{ijk}
7M'	0.17 ± 0.00^{abcd}	0.06 ± 0.00^{abcd}	0.06 ± 0.00^{ab}	0.63 ± 0.04^{abcdefghijk}	0.71 ± 0.01^{abcde}	0.27 ± 0.02^{abcdefghijkl}	0.45 ± 0.09^{ghi}	0.91 ± 0.02^{ab}
8G	0.12 ± 0.01^{fghijklmnopq}	0.07 ± 0.01^{abcde}	0.37 ± 0.00^{jklm}	0.57 ± 0.02^{opqrstuvwx}	0.55 ± 0.02^{lmnopq}	0.15 ± 0.01^{abcd}	0.53 ± 0.01^{hijk}	0.68 ± 0.02^{jkl}
8M'	0.26 ± 0.15^{ab}	0.08 ± 0.00^{abcdef}	0.55 ± 0.01^{abcdefg}	0.46 ± 0.03^{ijklmno}	0.24 ± 0.01^{bc}	0.19 ± 0.00^{abcdefg}	0.88 ± 0.01^{abcd}	0.62 ± 0.03^{fg}

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8M''	$0.09 \pm 0.00^{bcdefghijkl}$	0.06 ± 0.00^{abcd}	0.15 ± 0.00^{abcdefg}	0.52 ± 0.01^{klmnopq}	0.44 ± 0.08^{fghij}	0.19 ± 0.00^{abcdefg}	1.05 ± 0.02^{abcd}	0.46 ± 0.01^{c}
8P	$0.10 \pm 0.00^{cdefghijklmn}$	0.15 ± 0.00^{ghijk}	0.09 ± 0.00^{abcde}	$0.65 \pm 0.03^{abcdefghij}$	0.64 ± 0.05^{abcdef}	$0.40 \pm 0.00^{efghijklm}$	0.92 ± 0.07^{abcd}	0.73 ± 0.00^{mnopqrs}
9G'	$0.09 \pm 0.00^{abcdefghijk}$	$0.10 \pm 0.00^{abcdefghi}$	0.19 ± 0.01^{defgh}	$0.57 \pm 0.03^{nopqrstuvw}$	0.45 ± 0.02^{fghijk}	$0.21 \pm 0.00^{abcdefghi}$	0.76 ± 0.02^{abcde}	0.70 ± 0.00^{jklmn}
9P	$0.09 \pm 0.00^{bcdefghijkl}$	0.06 ± 0.00^{ab}	0.14 ± 0.01^{abcdefg}	$0.57 \pm 0.05^{mnopqrstuvw}$	0.27 ± 0.00^{bc}	$0.40 \pm 0.16^{efghijklm}$	0.61 ± 0.04^{klmnop}	0.81 ± 0.00^{abcdefgh}
10G	$0.12 \pm 0.01^{ghijklmnopq}$	0.08 ± 0.00^{abcdef}	0.47 ± 0.01^{mnopqrst}	$0.55 \pm 0.01^{mnopqrstu}$	0.28 ± 0.06^{bcd}	0.19 ± 0.01^{abcdefg}	0.47 ± 0.02^{ghi}	0.72 ± 0.01^{lmnopqr}
10G1	$0.09 \pm 0.00^{abcdefghijk}$	0.06 ± 0.00^{abcd}	0.56 ± 0.04^{abcdef}	0.53 ± 0.00^{lmnopqr}	0.53 ± 0.00^{jklmnop}	0.61 ± 0.06^{mnopq}	0.86 ± 0.08^{abcde}	0.64 ± 0.04^{ghi}
10G2	$0.09 \pm 0.00^{bcdefghijkl}$	0.09 ± 0.00^{abcdefgh}	0.65 ± 0.00^{abcde}	0.46 ± 0.04^{hijklmn}	0.43 ± 0.08^{fgh}	0.41 ± 0.08^{fghijklm}	0.71 ± 0.01^{pqrstu}	0.51 ± 0.03^{c}
11G1	$0.09 \pm 0.00^{abcdefghijkl}$	0.06 ± 0.00^{ab}	0.07 ± 0.00^{abc}	$0.55 \pm 0.09^{mnopqrstu}$	0.54 ± 0.00^{klmnop}	0.14 ± 0.05^{abcd}	0.78 ± 0.00^{abcde}	0.78 ± 0.01^{abcdef}
11G2	0.06 ± 0.00^{abcde}	0.06 ± 0.00^{ab}	0.22 ± 0.03^{fghi}	0.54 ± 0.02^{mnopqrst}	$0.52 \pm 0.00^{hijklmnop}$	0.14 ± 0.01^{abc}	0.41 ± 0.07^{efg}	0.95 ± 0.00^{a}
11G3	0.05 ± 0.00^{ab}	0.09 ± 0.00^{abcdefg}	0.39 ± 0.00^{klmn}	0.19 ± 0.01^{b}	0.58 ± 0.02^{nopqrs}	0.17 ± 0.00^{abcdef}	0.55 ± 0.04^{ijkl}	0.25 ± 0.01^{b}
11G4	0.08 ± 0.00^{abcdefgh}	0.08 ± 0.00^{abcdef}	0.25 ± 0.04^{ghij}	$0.58 \pm 0.02^{abcdefghijk}$	0.60 ± 0.02^{pqrst}	0.13 ± 0.01^{ab}	0.34 ± 0.02^{def}	0.62 ± 0.00^{fgh}
11G5	$0.11 \pm 0.00^{fghijklmnop}$	0.07 ± 0.00^{abcde}	0.32 ± 0.02^{ijkl}	$0.65 \pm 0.01^{abcdefghij}$	0.43 ± 0.08^{fghi}	0.18 ± 0.01^{abcdef}	0.80 ± 0.04^{abcde}	0.83 ± 0.03^{abcd}
12G	$0.08 \pm 0.00^{abcdefghijk}$	0.05 ± 0.00^{a}	0.55 ± 0.04^{abcdefg}	$0.55 \pm 0.01^{mnopqrstu}$	0.53 ± 0.02^{jklmnop}	0.15 ± 0.01^{abcd}	1.29 ± 0.03^{a}	0.80 ± 0.01^{abcdefg}
12G2	$0.10 \pm 0.01^{cdefghijklmn}$	0.06 ± 0.00^{abcd}	0.51 ± 0.02^{abcdefg}	0.46 ± 0.11^{ijklmno}	1.04 ± 0.01^{a}	0.18 ± 0.00^{abcdef}	0.55 ± 0.02^{ijkl}	0.78 ± 0.00^{abcdef}
12G3	$0.11 \pm 0.00^{fghijklmn}$	0.08 ± 0.00^{abcdef}	0.40 ± 0.00^{lmno}	0.67 ± 0.02^{abcdefg}	0.30 ± 0.00^{bcd}	0.18 ± 0.03^{abcdefg}	0.68 ± 0.12^{nopqrst}	0.56 ± 0.01^{de}
13M	$0.14 \pm 0.00^{abcdefghi}$	0.13 ± 0.06^{defghij}	0.73 ± 0.00^{abc}	0.48 ± 0.01^{jklmnop}	0.72 ± 0.01^{abcde}	0.17 ± 0.02^{abcde}	0.44 ± 0.05^{fgh}	0.70 ± 0.00^{klmno}
13M2	0.21 ± 0.00^{abcdef}	0.13 ± 0.02^{cdefghi}	0.81 ± 0.03^{ab}	0.53 ± 0.09^{lmnopqrs}	0.22 ± 0.00^{b}	0.51 ± 0.00^{lmnop}	0.58 ± 0.00^{jklmn}	0.82 ± 0.02^{abcde}
13M5	$0.12 \pm 0.00^{hijklmnopqr}$	0.06 ± 0.00^{abcd}	0.69 ± 0.02^{abc}	0.40 ± 0.05^{efghij}	0.57 ± 0.05^{mnopqr}	$0.33 \pm 0.01^{bcdefghijkl}$	0.69 ± 0.04^{opqrst}	0.77 ± 0.02^{abcde}
14P2	0.06 ± 0.00^{abcde}	0.04 ± 0.00^{a}	0.41 ± 0.01^{lmnop}	$0.57 \pm 0.02^{nopqrstuvw}$	0.46 ± 0.04^{fghijkl}	0.17 ± 0.02^{abcdef}	0.87 ± 0.01^{abcd}	1.10 ± 0.01^{a}
14P3	0.48 ± 0.01^{a}	0.06 ± 0.00^{ab}	0.60 ± 0.06^{abcde}	$0.65 \pm 0.13^{abcdefghij}$	0.78 ± 0.01^{abc}	0.18 ± 0.01^{abcdef}	1.20 ± 0.07^{abc}	0.75 ± 0.02^{rstuvw}
14P4	0.28 ± 0.00^{a}	0.15 ± 0.18^{hijk}	0.76 ± 0.01^{abc}	0.75 ± 0.00^{abcd}	0.77 ± 0.00^{abc}	0.19 ± 0.03^{abcdefg}	1.51 ± 0.05^{ab}	0.69 ± 0.02^{jklm}

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15G	0.12 ± 0.00^{klmnopqrs}	0.27 ± 0.01^{mn}	0.57 ± 0.06^{abcdefg}	0.91 ± 0.13^{ab}	0.75 ± 0.02^{abcd}	0.67 ± 0.01^{opq}	0.73 ± 0.01^{abcde}	0.75 ± 0.03^{qrstuvw}
15M1	0.16 ± 0.05^{abcdef}	0.04 ± 0.00^{a}	0.19 ± 0.03^{cdefgh}	0.17 ± 0.00^{b}	0.53 ± 0.00^{jklmnop}	0.17 ± 0.00^{abcde}	0.27 ± 0.00^{cd}	0.81 ± 0.01^{abcdefgh}
15M2	0.17 ± 0.00^{abcde}	0.25 ± 0.02^{lm}	0.67 ± 0.01^{abcd}	0.60 ± 0.06^{abcdefghij}	0.33 ± 0.01^{cde}	0.26 ± 0.06^{abcdefghijk}	0.62 ± 0.04^{klmnop}	0.81 ± 0.02^{abcdef}
15M3	0.22 ± 0.00^{abcd}	0.14 ± 0.01^{fghijk}	0.52 ± 0.02^{abcdefg}	1.05 ± 0.02^{ab}	0.54 ± 0.02^{klmnopq}	0.34 ± 0.03^{bcdefghijkl}	1.40 ± 0.02^{a}	0.79 ± 0.01^{abcdef}
15M4	0.11 ± 0.01^{fghijklmno}	0.06 ± 0.01^{abcd}	0.27 ± 0.04^{hijk}	0.43 ± 0.06^{fghijkl}	0.48 ± 0.05^{ghijklm}	0.17 ± 0.02^{abcdef}	0.16 ± 0.01^{b}	0.85 ± 0.02^{ab}
16G	0.13 ± 0.01^{lmnopqrst}	0.09 ± 0.00^{abcdefgh}	0.87 ± 0.01^{a}	0.69 ± 0.01^{abcdefg}	0.57 ± 0.05^{mnopqr}	0.23 ± 0.02^{abcdefghij}	0.81 ± 0.03^{abcde}	0.74 ± 0.01^{nopqrstu}
16M	0.16 ± 0.01^{abcde}	0.06 ± 0.00^{ab}	1.24 ± 0.28^{a}	0.64 ± 0.02^{abcdefghijk}	0.71 ± 0.09^{abcde}	0.22 ± 0.02^{abcdefghi}	0.48 ± 0.07^{ghij}	0.73 ± 0.01^{mnopqrst}
16M1	0.17 ± 0.00^{abcde}	0.05 ± 0.00^{a}	0.56 ± 0.05^{abcdef}	0.17 ± 0.03^{b}	0.41 ± 0.16^{efg}	0.21 ± 0.01^{abcdefghi}	0.20 ± 0.00^{bc}	0.59 ± 0.01^{def}
16M2	0.16 ± 0.00^{abcdefg}	0.08 ± 0.02^{abcdef}	0.04 ± 0.00^{a}	0.31 ± 0.03^{cde}	0.60 ± 0.02^{abcdef}	0.19 ± 0.00^{abcdefg}	0.55 ± 0.01^{ijkl}	0.71 ± 0.06^{klmnop}
16M3	0.10 ± 0.00^{bcdefghijkl}	0.10 ± 0.02^{abcdefghi}	1.16 ± 0.00^{a}	1.13 ± 0.23^{a}	0.59 ± 0.08^{opqrst}	0.18 ± 0.03^{abcdefg}	0.62 ± 0.01^{klmnopq}	0.77 ± 0.04^{abcde}
17G	0.15 ± 0.00^{abcdefgh}	0.12 ± 0.00^{bcdefghi}	0.33 ± 0.01^{ijkl}	0.66 ± 0.00^{abcdefghi}	0.85 ± 0.07^{a}	0.43 ± 0.05^{hijklmno}	0.32 ± 0.02^{de}	0.81 ± 0.01^{abcdefg}
17G1	0.10 ± 0.00^{efghijklmn}	0.13 ± 0.00^{efghijk}	1.22 ± 0.00^{a}	0.67 ± 0.04^{abcdefgh}	0.56 ± 0.04^{lmnopq}	0.15 ± 0.00^{abcd}	0.73 ± 0.00^{abcdef}	0.73 ± 0.01^{mnopqrs}
17G2	0.13 ± 0.01^{klmnopqrs}	0.32 ± 0.00^{n}	0.50 ± 0.04^{abcdefgh}	0.70 ± 0.02^{abcde}	0.78 ± 0.06^{abc}	0.21 ± 0.03^{abcdefghi}	1.42 ± 0.00^{a}	0.78 ± 0.04^{abcdef}
17G4	0.07 ± 0.00^{abcdef}	0.06 ± 0.00^{ab}	0.61 ± 0.00^{abcde}	0.39 ± 0.01^{efghij}	0.60 ± 0.00^{abcdef}	0.37 ± 0.05^{bcdefghijkl}	1.02 ± 0.00^{nopqrst}	0.97 ± 0.04^{ab}
17M	0.15 ± 0.00^{abcdefghi}	0.08 ± 0.02^{abcdef}	0.05 ± 0.00^{ab}	0.97 ± 0.02^{ab}	0.60 ± 0.01^{pqrst}	0.14 ± 0.00^{abc}	0.68 ± 0.05^{nopqrst}	0.90 ± 0.00^{ab}
17M2	0.23 ± 0.01^{ab}	0.08 ± 0.02^{abcdefg}	0.06 ± 0.00^{ab}	0.96 ± 0.00^{ab}	0.61 ± 0.01^{abcdef}	0.18 ± 0.02^{abcdefg}	0.58 ± 0.12^{jklm}	0.72 ± 0.00^{lmnopqrs}
18G	0.22 ± 0.00^{abc}	0.20 ± 0.02^{kl}	0.77 ± 0.01^{abc}	0.84 ± 0.02^{ab}	0.66 ± 0.04^{abcdefg}	0.36 ± 0.03^{bcdefghijkl}	1.27 ± 0.02^{a}	0.78 ± 0.01^{abcdef}
18M1	0.13 ± 0.01^{lmnopqrst}	0.07 ± 0.01^{abcd}	0.43 ± 0.02^{lmnopq}	0.53 ± 0.01^{lmnopqrs}	0.59 ± 0.00^{opqrs}	0.35 ± 0.00^{bcdefghijkl}	0.95 ± 0.01^{abcd}	0.84 ± 0.01^{abc}
18M2	0.06 ± 0.00^{abcd}	0.05 ± 0.00^{a}	0.37 ± 0.00^{jklm}	0.68 ± 0.04^{abcdefg}	0.49 ± 0.10^{ghijklmn}	0.14 ± 0.00^{abc}	0.76 ± 0.05^{abcde}	0.81 ± 0.00^{abcde}
Control without isolate	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.07 ± 0.00^{abc}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}

Values with different superscript letters on the same column are significantly different at $p < 0.05$.

3.4. Effect of Salinity Variation on the Growth of the 73 *Rhizobium* sp. Isolates

Generally, the optimal growth, measured by optical density at 620 nm, ranged from 0.44 ± 0.01 for isolate 17G4 to 1.05 ± 0.00 for isolate 5M at 1% NaCl, indicating more than two-fold difference in growth capacity under mild saline stress (Table 3). However, some isolates exhibited their highest growth at significantly higher salinity levels. For instance, isolate 6G reached 1.1 ± 0.01 at 2% NaCl, 10G2 exhibited a notable halotolerance with optimal growth values of 1.20 ± 0.04 at 6%, 1.39 ± 0.01 at 8%, and 1.19 ± 0.01 at 10% NaCl, maintaining over 90% of its maximum growth even at high salinity. In contrast, highly sensitive isolates such as 8P recorded only 0.08 ± 0.00 at 12% NaCl, underscoring a more than 14-fold difference in salt stress response compared to the most tolerant isolates.

Table 3. Growth of *Rhizobium* sp. isolates at different salinity levels in terms of optical density at 620 nm.

Isolates	NaCl 1%	NaCl 2%	NaCl 4%	NaCl 6%	NaCl 8%	NaCl 10%	NaCl 12%
1	0.60 ± 0.02^{ghij}	0.55 ± 0.03^{ijklm}	0.45 ± 0.01^{jklmnopq}	0.37 ± 0.02^{defghijk}	0.13 ± 0.01^{abcdefg}	0.17 ± 0.00^{abcdefghijklm}	0.39 ± 0.00^{pqr}
1G	0.61 ± 0.04^{hijk}	0.65 ± 0.04^{abcd}	0.50 ± 0.02^{abcd}	0.59 ± 0.01^{abcd}	0.26 ± 0.02^{ghijklmn}	0.18 ± 0.01^{bcdefghijklmn}	0.27 ± 0.03^{klmnop}
1G'	0.53 ± 0.02^{cd}	0.50 ± 0.00^{ef}	0.44 ± 0.02^{ghijklmn}	0.43 ± 0.05^{jklmnopqr}	0.18 ± 0.00^{abcdefghijk}	0.34 ± 0.02^{rs}	0.23 ± 0.01^{ghijklmno}
1G''	0.67 ± 0.02^{nopqrst}	0.55 ± 0.05^{ijklm}	0.41 ± 0.01^{defg}	0.38 ± 0.01^{efghijklm}	0.42 ± 0.00^{opqrs}	0.53 ± 0.01^{ab}	0.71 ± 0.05^{abc}
1M	0.89 ± 0.04^{ab}	0.76 ± 0.03^{ab}	0.67 ± 0.00^{abc}	0.54 ± 0.02^{abcdefgh}	0.34 ± 0.0 3^{lmnop}	0.31 ± 0.02^{pqrs}	0.27 ± 0.04^{lmnop}
1P	0.63 ± 0.02^{ijklmn}	0.55 ± 0.01^{hijkl}	0.42 ± 0.00^{efghijkl}	0.44 ± 0.02^{lmnopqrs}	0.13 ± 0.01^{abcdefg}	0.33 ± 0.00^{qrs}	0.24 ± 0.04^{ijklmno}
2	0.67 ± 0.03^{mnopqr}	0.58 ± 0.04^{lmnopq}	0.43 ± 0.00^{fghijklm}	0.47 ± 0.02^{opqrstuv}	0.22 ± 0.01^{cdefghijklm}	0.57 ± 0.01^{ab}	0.28 ± 0.04^{lmnop}
2G	0.74 ± 0.01^{abcdef}	0.64 ± 0.01^{abcd}	0.46 ± 0.00^{lmnopqrst}	0.45 ± 0.00^{nopqrstu}	0.59 ± 0.02^{a}	0.64 ± 0.02^{abc}	0.21 ± 0.02^{defghijklmn}
2M	0.62 ± 0.01^{hijkl}	0.57 ± 0.02^{klmno}	0.49 ± 0.02^{abcdef}	0.54 ± 0.06^{abcdefgh}	0.11 ± 0.00^{abcd}	0.22 ± 0.00^{ghijklmno}	0.39 ± 0.04^{pqr}
2M'	0.77 ± 0.05^{ab}	0.71 ± 0.05^{ab}	0.62 ± 0.08^{abc}	0.57 ± 0.06^{abcde}	0.72 ± 0.04^{a}	0.86 ± 0.24^{a}	0.79 ± 0.05^{ab}
2P'C'	0.89 ± 0.10^{ab}	0.71 ± 0.00^{abc}	0.45 ± 0.03^{hijklmnop}	0.40 ± 0.02^{ghijklmno}	0.18 ± 0.00^{abcdefghijk}	1.03 ± 0.00^{a}	0.24 ± 0.00^{hijklmno}
3G	0.81 ± 0.02^{ab}	0.61 ± 0.00^{abcdef}	0.4 ± 0.01^{efghij}	0.43 ± 0.05^{jklmnopqr}	0.22 ± 0.05^{cdefghijklm}	0.18 ± 0.01^{bcdefghijklmn}	0.31 ± 0.03^{mnopq}
3M'	0.69 ± 0.01^{abcdefgh}	0.67 ± 0.01^{abcd}	0.46 ± 0.01^{lmnopqrs}	0.38 ± 0.02^{efghijkl}	0.15 ± 0.01^{abcdefgh}	0.37 ± 0.05st	0.20 ± 0.01^{abcdefghijklm}
3M'''	0.86 ± 0.02^{ab}	0.64 ± 0.01^{abcd}	0.42 ± 0.01^{efghijk}	0.36 ± 0.05^{cdefgh}	0.44 ± 0.05^{pqrs}	0.13 ± 0.00^{abcdefg}	0.13 ± 0.02^{abcdefghi}

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4M1	0.57 ± 0.02^{defgh}	0.56 ± 0.04^{jklmn}	0.47 ± 0.00^{mnopqrstu}	0.46 ± 0.01^{nopqrstu}	0.08 ± 0.00^{ab}	0.19 ± 0.01^{defghijklmn}	0.08 ± 0.01^{a}
4M3	0.67 ± 0.01^{mnopqrs}	0.64 ± 0.03^{abcd}	0.52 ± 0.02^{abcd}	0.58 ± 0.09^{abcdef}	0.44 ± 0.07^{pqrs}	0.61 ± 0.01^{abc}	0.78 ± 0.01^{ab}
5G	0.92 ± 0.05^{a}	0.86 ± 0.04^{a}	0.46 ± 0.01^{lmnopqrs}	0.42 ± 0.04^{ijklmnopq}	0.25 ± 0.00^{efghijklmn}	0.25 ± 0.05^{ijklmnopq}	0.21 ± 0.01^{cdefghijklmn}
5M	1.05 ± 0.00^{a}	0.90 ± 0.00^{a}	0.58 ± 0.02^{abc}	0.39 ± 0.06^{efghijklm}	0.20 ± 0.00^{bcdefghijkl}	0.16 ± 0.01^{abcdefghij}	0.18 ± 0.01^{abcdefghijkl}
5M''	0.61 ± 0.00^{hijk}	0.56 ± 0.02^{ijklmn}	0.34 ± 0.01^{a}	0.33 ± 0.10^{bcde}	0.54 ± 0.02^{ab}	0.36 ± 0.00st	0.16 ± 0.00^{abcdefghijkl}
5M2'	0.76 ± 0.03^{abcd}	0.79 ± 0.04^{a}	0.74 ± 0.01^{a}	0.42 ± 0.00^{hijklmnop}	0.30 ± 0.04^{ijklmno}	0.51 ± 0.00^{ab}	0.17 ± 0.03^{abcdefghijkl}
5P	0.76 ± 0.02^{abcd}	0.5 ± 0.04^{ef}	0.39 ± 0.01^{bcde}	0.37 ± 0.04^{defghijk}	0.20 ± 0.00^{bcdefghijkl}	0.21 ± 0.01^{fghijklmno}	0.13 ± 0.00^{abcdefghi}
5P'	0.59 ± 0.00^{fghi}	0.48 ± 0.01^{de}	0.41 ± 0.01^{efgh}	0.38 ± 0.01^{efghijklm}	0.13 ± 0.01^{abcdefg}	0.11 ± 0.01^{abcde}	0.13 ± 0.00^{abcdefghi}
6G	0.67 ± 0.01^{mnopqrst}	1.11 ± 0.01^{a}	0.42 ± 0.02^{efghi}	0.36 ± 0.05^{cdefgh}	0.35 ± 0.02^{mnopq}	0.85 ± 0.02^{a}	0.25 ± 0.04^{ijklmno}
6G'	0.56 ± 0.02^{cdefg}	0.50 ± 0.00^{efg}	0.45 ± 0.01^{ijklmnopq}	0.50 ± 0.00^{abcdefgh}	0.17 ± 0.00^{abcdefghij}	0.22 ± 0.02^{ghijklmnop}	0.16 ± 0.01^{abcdefghijkl}
6G2	0.55 ± 0.02^{cdefg}	0.55 ± 0.00^{hijklm}	0.46 ± 0.00^{lmnopqrs}	0.29 ± 0.02^{b}	0.16 ± 0.00^{abcdefghi}	0.14 ± 0.00^{abcdefgh}	0.27 ± 0.24^{klmnop}
6G2'	0.67 ± 0.01^{mnopqrst}	0.64 ± 0.00^{abcd}	0.36 ± 0.01^{ab}	0.33 ± 0.02^{bcdef}	0.22 ± 0.01^{bcdefghijklm}	0.20 ± 0.02^{fghijklmno}	0.10 ± 0.00^{abcdef}
6G3	0.67 ± 0.03^{mnopqrs}	0.59 ± 0.01^{lmnop}	0.48 ± 0.01^{abcdefghi}	0.41 ± 0.00^{hijklmno}	0.31 ± 0.02^{jklmnop}	0.85 ± 0.00^{a}	0.66 ± 0.05^{ab}
6G4	0.77 ± 0.03^{abc}	0.57 ± 0.05^{klmno}	0.42 ± 0.01^{efghij}	0.31 ± 0.00^{bcd}	0.21 ± 0.07^{bcdefghijklm}	0.27 ± 0.00^{nopqr}	0.34 ± 0.01^{opq}
7M	0.72 ± 0.07^{abcdefg}	0.61 ± 0.00^{abcdef}	0.61 ± 0.00^{abc}	0.87 ± 0.00^{a}	0.75 ± 0.00^{a}	0.96 ± 0.00^{a}	0.58 ± 0.04st
7M'	0.77 ± 0.02^{abcd}	0.65 ± 0.01^{abcd}	0.39 ± 0.01^{bcde}	0.46 ± 0.04^{nopqrstu}	0.13 ± 0.01^{abcdefg}	0.22 ± 0.08^{ghijklmnop}	0.18 ± 0.07^{abcdefghijkl}
8G	0.65 ± 0.00^{klmnop}	0.42 ± 0.00^{c}	0.50 ± 0.02^{abcdef}	0.40 ± 0.02^{fghijklmn}	0.23 ± 0.02^{cdefghijklmn}	0.17 ± 0.00^{abcdefghijklm}	0.11 ± 0.00^{abcdefg}
8M'	0.57 ± 0.00^{defgh}	0.54 ± 0.00^{ghijk}	0.48 ± 0.01^{abcdefgh}	0.58 ± 0.01^{abcde}	0.14 ± 0.00^{abcdefg}	0.17 ± 0.00^{abcdefghijkl}	0.10 ± 0.00^{abcdef}
8M''	0.59 ± 0.02^{ghi}	0.44 ± 0.04 ^{cd}	0.61 ± 0.00^{abc}	0.68 ± 0.04^{a}	0.49 ± 0.02^{abcd}	0.79 ± 0.01 abc	0.59 ± 0.02 abc
8P	0.87 ± 0.00^{ab}	0.92 ± 0.00^{a}	0.44 ± 0.00^{hijklmno}	0.32 ± 0.03^{bcde}	0.21 ± 0.01^{bcdefghijkl}	0.17 ± 0.01^{abcdefghijkl}	0.08 ± 0.00^{ab}
9G'	0.73 ± 0.00^{abcdef}	0.68 ± 0.00^{abc}	0.49 ± 0.00^{abcdefg}	0.53 ± 0.07^{abcdefgh}	0.12 ± 0.00^{abcde}	0.08 ± 0.00^{a}	0.10 ± 0.01^{abcde}

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9P	0.51 ± 0.03^{c}	0.58 ± 0.00^{lmnop}	0.59 ± 0.03^{abc}	0.58 ± 0.01^{abcdef}	0.55 ± 0.01^{ab}	0.79 ± 0.08^{ab}	0.33 ± 0.03^{nopq}
10G	0.73 ± 0.02^{abcdefg}	0.55 ± 0.03^{hijkl}	0.44 ± 0.00^{ghijklmn}	0.45 ± 0.03^{nopqrstu}	0.12 ± 0.00^{abcdef}	0.16 ± 0.01^{abcdefghijk}	0.10 ± 0.00^{abcde}
10G1	0.70 ± 0.03^{abcdefg}	0.71 ± 0.01^{abc}	0.55 ± 0.03^{abcd}	0.43 ± 0.02^{jklmnopq}	0.32 ± 0.01^{klmnop}	0.65 ± 0.04^{abc}	0.42 ± 0.05^{qr}
10G2	0.63 ± 0.01^{ijklm}	0.22 ± 0.02^{a}	0.67 ± 0.01 ^{ab}	1.20 ± 0.04^{a}	1.30 ± 0.04^{a}	1.19 ± 0.01^{a}	1.17 ± 0.07^{a}
11G1	0.42 ± 0.00^{b}	0.42 ± 0.00^{c}	0.48 ± 0.01^{abcdefg}	0.52 ± 0.05^{abcdefg}	0.13 ± 0.02^{abcdefg}	0.19 ± 0.00^{defghijklmn}	0.09 ± 0.00^{abcde}
11G2	0.54 ± 0.01^{cde}	0.51 ± 0.01^{efgh}	0.46 ± 0.01^{klmnopqr}	0.57 ± 0.00^{abcdefg}	0.09 ± 0.01^{abc}	0.09 ± 0.00^{ab}	0.08 ± 0.01^{abc}
11G3	0.65 ± 0.00^{klmnop}	0.57 ± 0.04^{jklmn}	0.40 ± 0.00^{cdef}	0.60 ± 0.05^{abc}	0.26 ± 0.01^{fghijklmn}	0.17 ± 0.03^{abcdefghijklm}	0.47 ± 0.04^{rs}
11G4	0.66 ± 0.00^{lmnopqr}	0.62 ± 0.00^{abcde}	0.53 ± 0.00^{abcd}	0.45 ± 0.03^{nopqrstu}	0.18 ± 0.02^{abcdefghijk}	0.16 ± 0.00^{abcdefghijk}	0.21 ± 0.00^{bcdefghijklmn}
11G5	0.68 ± 0.06^{abcdefg}	0.68 ± 0.00^{abc}	0.70 ± 0.00^{ab}	0.69 ± 0.00^{a}	0.77 ± 0.03^{a}	0.96 ± 0.03 ^{a}	0.83 ± 0.03^{a}
12G	0.75 ± 0.00^{abcde}	0.57 ± 0.00^{jklmn}	0.52 ± 0.00^{abcd}	0.47 ± 0.02^{opqrstuv}	0.24 ± 0.00^{defghijklmn}	0.12 ± 0.01^{abcdef}	0.14 ± 0.01^{abcdefghijk}
12G2	0.63 ± 0.00^{ijklmno}	0.59 ± 0.01^{abcdef}	0.36 ± 0.01^{abc}	0.30 ± 0.01^{bc}	0.50 ± 0.05^{abc}	0.18 ± 0.00^{cdefghijklmn}	0.09 ± 0.01^{abcde}
12G3	0.45 ± 0.00^{b}	0.32 ± 0.00^{b}	0.44 ± 0.02^{fghijklm}	0.46 ± 0.00^{nopqrstu}	0.26 ± 0.02^{ghijklmn}	0.47 ± 0.02^{a}	0.61 ± 0.06 ^{ab}
13M	0.54 ± 0.01^{cdef}	0.53 ± 0.00^{fghij}	0.49 ± 0.01^{abcdefg}	0.52 ± 0.04^{abcdefg}	0.19 ± 0.05^{abcdefghijk}	0.25 ± 0.01^{jklmnopqr}	0.14 ± 0.00^{abcdefghij}
13M2	0.61 ± 0.04^{hijk}	0.60 ± 0.00^{abcdef}	0.65 ± 0.01^{abc}	0.61 ± 0.05^{ab}	0.28 ± 0.01^{hijklmno}	0.09 ± 0.01^{abc}	0.17 ± 0.01^{abcdefghijkl}
13M5	0.72 ± 0.01^{abcdefg}	0.79 ± 0.00^{a}	0.68 ± 0.01^{ab}	0.60 ± 0.07^{abc}	1.39 ± 0.01^{a}	0.46 ± 0.2^{a}	0.48 ± 0.00^{rs}
14P2	0.67 ± 0.00^{mnopqrs}	0.63 ± 0.00^{abcde}	0.63 ± 0.02^{abc}	0.63 ± 0.05^{ab}	0.75 ± 0.03^{a}	0.57 ± 0.03^{ab}	0.70 ± 0.03^{abc}
14P3	0.61 ± 0.02^{hijk}	0.55 ± 0.01^{hijklm}	0.64 ± 0.00^{abcd}	0.46 ± 0.01^{nopqrstu}	0.37 ± 0.05^{nopqr}	0.70 ± 0.01^{abc}	0.21 ± 0.01^{cdefghijklmn}
14P4	0.59 ± 0.03^{ghi}	0.65 ± 0.02^{abcd}	0.56 ± 0.00^{abc}	0.50 ± 0.04^{abcdefgh}	0.31 ± 0.04^{jklmnop}	0.15 ± 0.00^{abcdefgh}	0.43 ± 0.04^{qr}
15G	0.68 ± 0.00^{nopqrst}	0.63 ± 0.02^{abcd}	0.64 ± 0.02^{abcd}	0.54 ± 0.05^{abcdefghi}	0.41 ± 0.04^{opqrs}	0.73 ± 0.03^{abc}	0.28 ± 0.03^{lmnop}
15M1	0.60 ± 0.01^{ghij}	0.53 ± 0.00^{fghjij}	0.45 ± 0.01^{hijklmnop}	0.49 ± 0.03^{qrstuvwx}	0.16 ± 0.01^{abcdefgh}	0.22 ± 0.00^{ghijklmnop}	0.11 ± 0.00^{abcdefg}
15M2	0.59 ± 0.00^{ghij}	0.58 ± 0.00^{lmnop}	0.52 ± 0.00^{abcd}	0.54 ± 0.02^{abcdefghi}	0.11 ± 0.00^{abcd}	0.20 ± 0.00^{fghijklmno}	0.17 ± 0.00^{abcdefghijkl}
15M3	0.71 ± 0.00^{abcdefg}	0.63 ± 0.01^{abcde}	0.56 ± 0.05^{abcd}	0.48 ± 0.05^{pqrstuvw}	0.11 ± 0.00^{abcd}	0.19 ± 0.01^{defghijklmn}	0.12 ± 0.01^{abcdefgh}

Continued

15M4	0.86 ± 0.06^{a}	0.75 ± 0.02^{ab}	$0.47 \pm 0.00^{mnopqrstu}$	0.32 ± 0.03^{bcde}	0.11 ± 0.01^{abcd}	0.29 ± 0.00^{opqrs}	0.25 ± 0.04^{ijklmno}
16G	0.64 ± 0.01^{jklmnop}	0.64 ± 0.02^{abcde}	0.51 ± 0.02^{abcd}	0.45 ± 0.03^{mnopqrst}	0.14 ± 0.07^{abcdefgh}	0.10 ± 0.01^{abcd}	$0.18 \pm 0.00^{abcdefghijkl}$
16M	0.52 ± 0.03^{c}	0.51 ± 0.01^{efghi}	$0.48 \pm 0.00^{abcdefghi}$	$0.44 \pm 0.00^{klmnopqrs}$	$0.24 \pm 0.01^{defghijklmn}$	$0.24 \pm 0.01^{ijklmnopq}$	0.24 ± 0.01^{hijklmno}
16M1	0.65 ± 0.00^{klmnopq}	0.71 ± 0.00^{abc}	0.63 ± 0.01^{abc}	0.41 ± 0.00^{hijklmno}	0.15 ± 0.00^{abcdefgh}	$0.22 \pm 0.01^{ghijklmno}$	0.09 ± 0.00^{abcd}
16M2	0.57 ± 0.01^{defgh}	0.63 ± 0.01^{abcde}	0.50 ± 0.00^{abcde}	$0.43 \pm 0.01^{jklmnopqr}$	0.14 ± 0.01^{abcdefg}	0.26 ± 0.00^{lmnopqr}	0.10 ± 0.00^{abcdef}
16M3	0.73 ± 0.00^{abcdefg}	0.61 ± 0.04^{abcdef}	0.37 ± 0.00^{abcd}	0.36 ± 0.02^{cdefghi}	0.12 ± 0.02^{abcdef}	$0.20 \pm 0.00^{efghijklmno}$	0.42 ± 0.55^{qr}
17G	0.87 ± 0.00^{a}	0.67 ± 0.02^{abcd}	$0.46 \pm 0.00^{jklmnopqr}$	0.63 ± 0.00^{ab}	$0.20 \pm 0.00^{bcdefghijk}$	$0.22 \pm 0.00^{ghijklmnop}$	$0.22 \pm 0.00^{fghijklmno}$
17G1	0.68 ± 0.00^{abcdefgh}	0.59 ± 0.03^{lmnopq}	0.59 ± 0.01^{ab}	0.51 ± 0.03^{abcdefg}	$0.18 \pm 0.00^{abcdefghijk}$	0.27 ± 0.01^{mnopqr}	$0.22 \pm 0.00^{efghijklmno}$
17G2	0.61 ± 0.04^{hijkl}	0.72 ± 0.02^{abc}	0.42 ± 0.00^{efghijkl}	$0.40 \pm 0.00^{fghijklmn}$	0.31 ± 0.03^{jklmnop}	0.26 ± 0.00^{lmnopqr}	$0.16 \pm 0.01^{abcdefghijkl}$
17G4	0.44 ± 0.01^{b}	0.42 ± 0.07^{c}	$0.47 \pm 0.03^{mnopqrstu}$	0.34 ± 0.06^{bcdefg}	0.11 ± 0.01^{abcde}	0.45 ± 0.01^{ab}	$0.18 \pm 0.00^{abcdefghijkl}$
17M	0.57 ± 0.01^{defgh}	0.64 ± 0.01^{abc}	0.72 ± 0.00^{ab}	0.55 ± 0.00^{abcdefgh}	$0.23 \pm 0.00^{cdefghijklm}$	0.26 ± 0.01^{klmnopqr}	0.12 ± 0.00^{abcdefgh}
17M2	0.6 ± 0.01^{ghij}	0.50 ± 0.00^{efg}	0.50 ± 0.02^{abcd}	0.37 ± 0.01^{defghij}	0.34 ± 0.04^{lmnop}	$0.19 \pm 0.00^{defghijklmn}$	$0.18 \pm 0.01^{abcdefghijkl}$
18G	0.51 ± 0.01^{c}	0.54 ± 0.00^{ghijk}	$0.48 \pm 0.03^{abcdefghi}$	0.56 ± 0.04^{abcdefg}	0.37 ± 0.02^{nopqr}	0.83 ± 0.03^{a}	0.41 ± 0.03^{qr}
18M1	0.59 ± 0.01^{efghi}	0.53 ± 0.00^{fghijk}	$0.47 \pm 0.01^{abcdefghi}$	0.52 ± 0.07^{abcdefgh}	$0.24 \pm 0.03^{defghijklmn}$	$0.15 \pm 0.03^{abcdefghi}$	$0.13 \pm 0.00^{abcdefghij}$
18M2	0.52 ± 0.00^{cd}	0.45 ± 0.00^{cd}	0.50 ± 0.00^{abcd}	0.58 ± 0.01^{abcdef}	0.27 ± 0.04^{ghijklmn}	$0.23 \pm 0.01^{hijklmnop}$	0.25 ± 0.05^{jklmno}
Control without isolate	0.27 ± 0.00^{a}	0.59 ± 0.00^{lmnopq}	0.46 ± 0.02^{lmnopqrs}	0.07 ± 0.00^{a}	0.06 ± 0.00^{a}	$0.19 \pm 0.24^{defghijklmn}$	0.10 ± 0.00^{abcdef}

Values with different superscript letters on the same column are significantly different at $p < 0.05$.

3.5. Effect of Temperature Variation on the Growth of the 73 *Rhizobium* sp. Isolates

As shown in Table 4, most isolates showed better growth at 30˚C, with OD values ranging from 0.50 ± 0.06 for isolate 5P to 0.84 ± 0.17 for isolate 2M, representing a 68% increase between the lowest and highest performance at this temperature. However, several isolates showed significantly ($p < 0.05$) high growth at other temperatures. Isolates 2M (1.06 ± 0.00) and 4M1 (1.12 ± 0.02) reached optimal

Table 4. Growth of *Rhizobium* sp. isolates at different temperatures in terms of optical density 620 nm

Isolates	15˚C	20˚C	25˚C	30˚C	35˚C	40˚C	45˚C
1	0.33 ± 0.00^{b}	0.53 ± 0.01^{abcd}	0.57 ± 0.01^{cdefg}	$0.75 \pm 0.01^{abcdefghi}$	0.76 ± 0.00^{abcde}	0.81 ± 0.00^{abcde}	0.28 ± 0.00^{cdef}
1G	0.80 ± 0.01^{abcdef}	0.85 ± 0.02^{ab}	0.62 ± 0.00^{jklmnop}	$0.77 \pm 0.03^{abcdefghi}$	0.82 ± 0.00^{abcd}	0.78 ± 0.00^{abcdef}	0.72 ± 0.00^{ab}
1G'	0.69 ± 0.00^{nopqrstu}	0.55 ± 0.02^{abcde}	0.60 ± 0.01^{ghijkl}	$0.74 \pm 0.00^{abcdefghi}$	0.28 ± 0.03^{abcd}	0.61 ± 0.03^{kl}	0.28 ± 0.00^{bcde}
1G"	0.76 ± 0.04^{abcde}	0.36 ± 0.01^{bcdefg}	0.63 ± 0.01^{lmnopqr}	$0.66 \pm 0.00^{ijklmnopqr}$	0.20 ± 0.00^{a}	0.77 ± 0.03^{abcdefg}	0.64 ± 0.02^{abcdefg}
1M	0.85 ± 0.04^{abc}	0.68 ± 0.00^{abc}	0.69 ± 0.00^{abcdefg}	0.78 ± 0.01^{abcdefg}	0.70 ± 0.04^{abcde}	0.44 ± 0.04^{ghij}	0.21 ± 0.00^{a}
1P	0.68 ± 0.00^{lmnopqr}	0.40 ± 0.00^{efghijkl}	0.62 ± 0.03^{jklmnop}	$0.67 \pm 0.00^{ijklmnopqrs}$	0.76 ± 0.02^{abcd}	0.26 ± 0.00^{abc}	0.64 ± 0.01^{abcdefgh}
2	0.75 ± 0.01^{abcdefgh}	0.38 ± 0.00^{cdefghij}	0.71 ± 0.01^{abcdef}	$0.72 \pm 0.00^{nopqrstuvwx}$	0.50 ± 0.07^{hijklmno}	0.78 ± 0.00^{abcdef}	0.72 ± 0.00^{ab}
2G	0.68 ± 0.00^{lmnopqr}	0.60 ± 0.02^{abcd}	0.60 ± 0.00^{fghijkl}	$0.66 \pm 0.00^{ijklmnopqr}$	0.69 ± 0.02^{abcde}	0.74 ± 0.02^{abcdef}	0.64 ± 0.02^{abcdefg}
2M	0.15 ± 0.00^{a}	1.06 ± 0.00^{a}	0.56 ± 0.00^{bcdef}	0.77 ± 0.00^{abcdefgh}	1.20 ± 0.02^{a}	0.36 ± 0.00^{cdefghi}	0.67 ± 0.01^{abc}
2M'	0.60 ± 0.15^{hi}	0.45 ± 0.01^{lmn}	0.60 ± 0.05^{ghijkl}	0.84 ± 0.17^{abc}	0.38 ± 0.25^{efg}	0.74 ± 0.03^{nopq}	0.47 ± 0.09^{lm}
2P'C'	$0.70 \pm 0.00^{abcdefghijk}$	0.35 ± 0.02^{bcdefg}	0.76 ± 0.00^{a}	$0.67 \pm 0.06^{jklmnopqrs}$	0.71 ± 0.01^{abcdef}	0.87 ± 0.01^{ab}	0.56 ± 0.02^{qrst}
3G	0.82 ± 0.01^{abcdef}	0.49 ± 0.02^{mno}	$0.67 \pm 0.00^{abcdefghij}$	$0.66 \pm 0.09^{ijklmnopqr}$	0.74 ± 0.00^{abcdef}	0.75 ± 0.02^{abcdef}	0.26 ± 0.01^{bcd}
3M'	0.61 ± 0.00^{hijk}	0.33 ± 0.03^{bcd}	0.58 ± 0.01^{cdefgh}	0.41 ± 0.18^{a}	0.41 ± 0.05^{fghi}	0.81 ± 0.03^{abcde}	0.26 ± 0.00^{bcd}
3M'''	0.33 ± 0.02^{b}	0.45 ± 0.02^{klmn}	0.61 ± 0.04^{ghijklmn}	0.61 ± 0.02^{defghijk}	0.66 ± 0.03^{abcdefg}	1.20 ± 0.03^{a}	0.66 ± 0.02^{abcdef}
4M1	$0.70 \pm 0.00^{nopqrstuvw}$	1.12 ± 0.02^{a}	0.63 ± 0.02^{klmnopqr}	$0.65 \pm 0.00^{hijklmnopq}$	0.42 ± 0.07^{fghij}	0.36 ± 0.07^{cdefghi}	0.34 ± 0.00^{hi}
4M3	0.45 ± 0.02^{c}	0.70 ± 0.00^{ab}	0.61 ± 0.00^{hijklmn}	$0.77 \pm 0.03^{abcdefghi}$	0.77 ± 0.01^{abcd}	0.85 ± 0.04^{abcde}	0.65 ± 0.02^{abcdefg}
5G	0.43 ± 0.03^{c}	0.62 ± 0.00^{abcd}	0.68 ± 0.00^{abcdefgh}	0.83 ± 0.01^{abcde}	0.68 ± 0.01^{abcde}	0.78 ± 0.02^{abcdef}	0.36 ± 0.00^{ij}
5M	0.75 ± 0.01^{abcdefg}	0.41 ± 0.01^{efghijkl}	0.58 ± 0.01^{defgh}	$0.68 \pm 0.00^{jklmnopqrstu}$	0.52 ± 0.03^{ijklmnop}	0.87 ± 0.00^{abc}	0.31 ± 0.00^{efgh}
5M"	$0.73 \pm 0.00^{abcdefghij}$	0.70 ± 0.01^{ab}	0.66 ± 0.03^{opqrstuv}	$0.71 \pm 0.00^{mnopqrstuvwx}$	0.42 ± 0.04^{fghij}	0.83 ± 0.01^{abcde}	0.38 ± 0.00^{jk}
5M2'	0.74 ± 0.02^{abcdefgh}	0.65 ± 0.00^{abc}	$0.66 \pm 0.00^{abcdefghij}$	$0.64 \pm 0.02^{ghijklmnop}$	1.17 ± 0.05^{a}	0.85 ± 0.01^{abcde}	0.68 ± 0.01^{ab}
5P	0.55 ± 0.00^{fgh}	0.33 ± 0.01^{bcd}	$0.67 \pm 0.00^{abcdefghi}$	0.50 ± 0.06^{bc}	0.22 ± 0.01^{a}	0.78 ± 0.01^{abcdef}	0.75 ± 0.02^{a}

Continued

5P'	0.74 ± 0.04^{abcdefghi}	0.30 ± 0.00^{ab}	0.55 ± 0.01^{abcde}	0.69 ± 0.00^{klmnopqrstuv}	0.52 ± 0.02^{hijklmnop}	0.72 ± 0.04^{lmnop}	0.73 ± 0.01^{a}
6G	0.74 ± 0.00^{abcdefghi}	0.33 ± 0.01^{bcd}	0.69 ± 0.00^{abcdefgh}	0.66 ± 0.00^{ijklmnopqrs}	0.61 ± 0.01^{abcdef}	0.30 ± 0.02^{abcdef}	0.28 ± 0.00^{cdef}
6G'	0.76 ± 0.05^{abcde}	0.63 ± 0.00^{abc}	0.68 ± 0.02^{abcdefghi}	0.73 ± 0.05^{abcdefghi}	0.65 ± 0.00^{abcdef}	0.26 ± 0.01^{abc}	0.63 ± 0.00^{abcdef}
6G2	0.84 ± 0.01^{abcde}	0.35 ± 0.00^{bcdef}	0.64 ± 0.01^{lmnopqrs}	0.72 ± 0.00^{abcdefghijk}	0.72 ± 0.02^{abcdef}	0.26 ± 0.00^{abc}	0.25 ± 0.01^{bc}
6G2'	0.62 ± 0.01^{ijkl}	0.69 ± 0.00 ^{abc}	0.71 ± 0.00^{abcdef}	0.69 ± 0.03^{klmnopqrstuv}	0.64 ± 0.01^{abcdef}	0.22 ± 0.00^{ab}	0.34 ± 0.00^{hi}
6G3	0.84 ± 0.00^{abcd}	0.35 ± 0.00^{bcdefg}	0.70 ± 0.00^{abcdefg}	0.90 ± 0.00^{a}	0.29 ± 0.02^{abcde}	0.27 ± 0.04^{abcd}	0.30 ± 0.00^{def}
6G4	0.52 ± 0.02^{ef}	0.36 ± 0.00^{bcdefghi}	0.67 ± 0.02^{abcdefghi}	0.73 ± 0.00^{abcdefghi}	0.49 ± 0.02^{hijklm}	0.33 ± 0.01^{abcdefg}	0.67 ± 0.01^{abcd}
7M	0.53 ± 0.03^{efg}	0.56 ± 0.00^{abcde}	0.68 ± 0.01^{abcdefgh}	0.65 ± 0.00^{hijklmnopq}	0.26 ± 0.00^{abc}	0.41 ± 0.03^{fghij}	0.57 ± 0.02^{rstu}
7M'	0.67 ± 0.00^{lmnopq}	0.36 ± 0.00^{bcdefgh}	0.73 ± 0.00^{abc}	0.64 ± 0.01^{ghijklmno}	0.76 ± 0.00^{abcd}	0.35 ± 0.00^{bcdefgh}	0.46 ± 0.01^{lm}
8G	0.69 ± 0.01^{nopqrst}	0.35 ± 0.00^{bcdefg}	0.54 ± 0.04^{abc}	0.59 ± 0.12^{defghi}	0.25 ± 0.05^{abc}	0.25 ± 0.01^{abc}	0.29 ± 0.01^{def}
8M'	0.62 ± 0.00^{ijklm}	0.41 ± 0.03^{ghijkl}	0.59 ± 0.01^{fghij}	0.65 ± 0.01^{hijklmnopq}	0.25 ± 0.03^{abc}	0.27 ± 0.00^{abcd}	0.34 ± 0.00^{ghi}
8M''	0.57 ± 0.00^{fghi}	0.36 ± 0.02^{bcdefghi}	0.63 ± 0.02^{klmnopqr}	0.59 ± 0.01^{defghi}	0.34 ± 0.06^{bcdef}	0.41 ± 0.04^{fghij}	0.58 ± 0.00^{stuvw}
8P	0.77 ± 0.02^{abcd}	0.39 ± 0.02^{defghijk}	0.72 ± 0.00^{abcde}	0.72 ± 0.00^{abcdefghijk}	0.62 ± 0.02^{abcdef}	1.08 ± 0.01^{a}	0.48 ± 0.00^{mn}
9G'	0.54 ± 0.02^{efg}	0.39 ± 0.02^{defghijk}	0.70 ± 0.00^{abcdefg}	0.66 ± 0.01^{ijklmnopqr}	0.75 ± 0.01^{abcde}	0.66 ± 0.02^{lmno}	0.44 ± 0.01^{l}
9P	0.76 ± 0.02^{abcde}	0.34 ± 0.00^{bcde}	0.61 ± 0.00^{ghijklm}	0.68 ± 0.00^{jklmnopqrst}	0.38 ± 0.01^{defg}	0.49 ± 0.11^{jk}	0.67 ± 0.01^{abc}
10G	0.66 ± 0.00^{jklmno}	0.60 ± 0.04^{abcde}	0.74 ± 0.00 ^{ab}	0.84 ± 0.02^{abcd}	0.41 ± 0.00^{fgh}	0.82 ± 0.01^{abcde}	0.40 ± 0.00^{k}
10G1	0.69 ± 0.00^{nopqrstuv}	0.40 ± 0.08^{efghijkl}	0.58 ± 0.00^{cdefgh}	0.70 ± 0.00^{klmnopqrstuv}	1.23 ± 0.04^{a}	0.30 ± 0.00^{abcdef}	0.68 ± 0.01^{ab}
10G2	0.79 ± 0.01^{abcde}	0.67 ± 0.00^{abc}	0.61 ± 0.00^{ghijklmn}	0.48 ± 0.00^{ab}	0.57 ± 0.05^{lmnopqr}	0.31 ± 0.00^{abcdef}	0.56 ± 0.01^{pqrst}
11G1	0.32 ± 0.01^{b}	0.26 ± 0.00^{a}	0.70 ± 0.02^{abcdef}	0.61 ± 0.00^{defghijkl}	0.61 ± 0.09^{abcdefg}	0.76 ± 0.00^{abcdefg}	0.30 ± 0.00^{efgh}
11G2	0.86 ± 0.00 ^{a}	0.32 ± 0.04^{abc}	0.64 ± 0.00^{lmnopqrs}	0.66 ± 0.00^{ijklmnopqr}	0.36 ± 0.03^{cdef}	0.34 ± 0.00^{bcdefgh}	0.34 ± 0.01^{ghi}
11G3	0.66 ± 0.03^{jklmno}	0.59 ± 0.00^{abcde}	0.52 ± 0.01^{a}	0.64 ± 0.00^{fghijklmno}	0.26 ± 0.01^{abc}	0.31 ± 0.03^{abcdef}	0.48 ± 0.01^{lm}

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11G4	0.85 ± 0.02^{ab}	0.60 ± 0.01^{abcd}	0.63 ± 0.00^{lmnopqrs}	0.73 ± 0.01^{abcdefghij}	0.68 ± 0.01^{abcdef}	0.28 ± 0.00^{abcdef}	0.63 ± 0.00^{abcde}
11G5	0.75 ± 0.00^{abcdefgh}	0.65 ± 0.00 a^{bc}	0.59 ± 0.11^{fghijk}	0.80 ± 0.02^{abcdef}	0.72 ± 0.02^{abcdef}	0.65 ± 0.01^{lmn}	0.62 ± 0.00^{abcdef}
12G	0.44 ± 0.02^{c}	0.91 ± 0.00^{a}	0.69 ± 0.00^{abcdefgh}	0.82 ± 0.01^{abcdef}	0.66 ± 0.03^{abcdefg}	0.95 ± 0.00^{a}	0.30 ± 0.02^{defg}
12G2	0.33 ± 0.00^{b}	0.69 ± 0.00^{abc}	0.66 ± 0.00^{abcdefghij}	0.87 ± 0.03^{ab}	0.63 ± 0.00^{abcdef}	0.87 ± 0.02^{abc}	0.56 ± 0.00^{pqrst}
12G3	0.49 ± 0.04 ^{cde}	0.53 ± 0.01^{opq}	0.63 ± 0.01^{jklmnopq}	0.56 ± 0.07^{bcdefg}	0.23 ± 0.01^{ab}	0.72 ± 0.01^{lmnop}	0.28 ± 0.00^{bcde}
13M	0.75 ± 0.02^{abcdef}	0.58 ± 0.01^{abcde}	0.66 ± 0.00^{abcdefghij}	0.70 ± 0.00^{lmnopqrstuvw}	0.42 ± 0.16^{fghij}	0.35 ± 0.06^{bcdefgh}	0.38 ± 0.00^{jk}
13M2	0.72 ± 0.01^{abcdefghij}	0.58 ± 0.04^{abcde}	0.54 ± 0.03^{abcd}	0.76 ± 0.05^{abcdefghi}	0.74 ± 0.04^{abcde}	0.49 ± 0.34^{jk}	0.30 ± 0.02^{efgh}
13M5	0.79 ± 0.00^{abcdef}	0.34 ± 0.01^{bcde}	0.66 ± 0.02^{opqrstuvw}	0.73 ± 0.00^{abcdefghi}	0.84 ± 0.00^{ab}	0.23 ± 0.00^{ab}	0.24 ± 0.01 ^{ab}
14P2	0.65 ± 0.03^{jklmn}	0.35 ± 0.00^{bcdefg}	0.71 ± 0.01^{abcdef}	0.77 ± 0.00^{abcdefgh}	0.41 ± 0.00^{fghi}	0.31 ± 0.00^{abcdef}	0.66 ± 0.00^{abcde}
14P3	0.46 ± 0.00^{cd}	0.37 ± 0.03^{cdefghij}	0.70 ± 0.01^{abcdefgh}	0.61 ± 0.00^{defghijk}	0.69 ± 0.10^{abcde}	0.62 ± 0.06 ^{lm}	0.63 ± 0.00^{abcdef}
14P4	0.67 ± 0.03^{klmnop}	0.82 ± 0.02^{a}	0.73 ± 0.03^{abcd}	0.66 ± 0.01^{ijklmnopqr}	0.73 ± 0.05^{abcdef}	0.76 ± 0.04^{abcdefg}	0.62 ± 0.01^{abcdef}
15G	0.52 ± 0.02^{def}	0.50 ± 0.06^{nop}	0.61 ± 0.00^{hijklmn}	0.63 ± 0.05^{efghijklm}	0.50 ± 0.13^{hijklmn}	0.78 ± 0.01^{abcdef}	0.59 ± 0.02^{tuvwx}
15M1	0.51 ± 0.08^{def}	0.33 ± 0.00^{bcd}	0.66 ± 0.01^{abcdefghijk}	0.68 ± 0.00^{jklmnopqrst}	0.83 ± 0.07^{abc}	0.36 ± 0.00^{cdefghi}	0.56 ± 0.02^{qrst}
15M2	0.75 ± 0.05^{abcdef}	0.37 ± 0.01^{cdefghi}	0.62 ± 0.05^{ijklmno}	0.55 ± 0.13^{bcdef}	0.88 ± 0.01^{a}	0.46 ± 0.15^{hij}	0.60 ± 0.00^{abcdef}
15M3	0.78 ± 0.00^{abcde}	0.35 ± 0.01^{bcdefg}	0.66 ± 0.00^{opqrstuvw}	0.62 ± 0.00^{defghijklm}	0.68 ± 0.02^{abcdef}	0.71 ± 0.01^{lmnop}	0.68 ± 0.00^{ab}
15M4	0.66 ± 0.00^{jklmn}	0.55 ± 0.07^{abcde}	0.53 ± 0.01^{ab}	0.78 ± 0.03^{abcdefg}	0.83 ± 0.01^{abcd}	0.23 ± 0.00^{ab}	0.55 ± 0.00^{opqrs}
16G	0.60 ± 0.00^{hij}	0.31 ± 0.00^{abc}	0.63 ± 0.01^{klmnopqr}	0.63 ± 0.02^{fghijklmn}	0.73 ± 0.01^{abcdef}	0.26 ± 0.01^{abc}	0.56 ± 0.02^{pqrst}
16M	0.51 ± 0.06^{def}	0.35 ± 0.00^{bcdefg}	0.64 ± 0.02^{lmnopqrst}	0.57 ± 0.01^{cdefgh}	0.55 ± 0.02^{klmnopq}	0.21 ± 0.01^{a}	0.61 ± 0.00^{abcdef}
16M1	0.72 ± 0.02^{abcdefghijk}	0.41 ± 0.05^{fghijkl}	0.52 ± 0.02^{a}	0.66 ± 0.03^{ijklmnopqr}	0.76 ± 0.00^{abcd}	0.39 ± 0.02^{defghij}	0.63 ± 0.01^{abcdef}
16M2	0.76 ± 0.01^{abcde}	0.35 ± 0.00^{bcdef}	0.72 ± 0.01^{abcde}	0.60 ± 0.11^{defghij}	0.77 ± 0.03^{abcd}	0.48 ± 0.02^{jk}	0.35 ± 0.01^{ij}
16M3	0.75 ± 0.01^{abcde}	0.35 ± 0.00^{bcdefg}	0.64 ± 0.00^{mnopqrstu}	0.71 ± 0.00^{mnopqrstuvwx}	0.61 ± 0.09^{abcdef}	0.31 ± 0.01^{abcdef}	0.53 ± 0.02^{opqr}

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17G	0.76 ± 0.01^{abcde}	0.44 ± 0.00^{jklmn}	0.65 ± 0.00^{nopqrstu}	$0.71 \pm 0.00^{mnopqrstuvwx}$	0.88 ± 0.09^{a}	0.66 ± 0.03^{lmno}	0.58 ± 0.00^{stuvw}
17G1	0.68 ± 0.00^{mnopqrs}	0.43 ± 0.01^{ijklm}	0.61 ± 0.00^{hijklmn}	0.57 ± 0.00^{cdefgh}	0.61 ± 0.07^{mnopqrs}	0.30 ± 0.00^{abcdef}	0.56 ± 0.01^{pqrst}
17G2	0.31 ± 0.01^{b}	0.42 ± 0.01^{hijklm}	0.61 ± 0.01^{hijklmn}	$0.74 \pm 0.00^{abcdefghi}$	0.25 ± 0.01^{abc}	0.81 ± 0.00^{abcde}	0.57 ± 0.01^{stuv}
17G4	$0.73 \pm 0.03^{abcdefghij}$	0.48 ± 0.07^{mno}	0.58 ± 0.00^{efghi}	$0.71 \pm 0.04^{nopqrstuvwx}$	0.55 ± 0.17^{lmnopq}	0.29 ± 0.00^{abcdef}	0.56 ± 0.03^{pqrst}
17M	0.86 ± 0.01^{a}	0.63 ± 0.01^{abc}	$0.67 \pm 0.02^{abcdefghi}$	$0.70 \pm 0.02^{mnopqrstuvwx}$	0.44 ± 0.04^{fghijk}	0.47 ± 0.03^{ij}	0.51 ± 0.00^{no}
17M2	0.59 ± 0.00^{ghi}	0.74 ± 0.00^{a}	$0.64 \pm 0.01^{mnopqrstu}$	0.54 ± 0.05^{bcd}	0.53 ± 0.02^{jklmnop}	0.28 ± 0.01^{abcde}	0.52 ± 0.00^{op}
18G	0.29 ± 0.03^{b}	0.59 ± 0.03^{abcdef}	0.64 ± 0.01^{lmnopqrs}	0.89 ± 0.00^{a}	0.48 ± 0.14^{ghijkl}	0.86 ± 0.02^{abcd}	0.32 ± 0.01^{fgh}
18M1	0.65 ± 0.03^{jklmn}	0.35 ± 0.00^{bcdefg}	0.61 ± 0.01^{ghijklm}	0.54 ± 0.03^{bcde}	0.82 ± 0.04^{abcd}	0.67 ± 0.02^{lmno}	0.59 ± 0.00^{abcdef}
18M2	0.76 ± 0.04^{abcd}	0.41 ± 0.00^{fghijkl}	0.61 ± 0.00^{hijklmn}	$0.66 \pm 0.00^{ijklmnopqr}$	0.21 ± 0.00^{a}	0.40 ± 0.08^{efghij}	0.53 ± 0.00^{opq}
Control without isolate	0.16 ± 0.00^{a}	0.71 ± 0.02^{ab}	0.63 ± 0.01^{lmnopqr}	$0.64 \pm 0.00^{fghijklmno}$	0.74 ± 0.03^{abcde}	0.66 ± 0.01^{lmno}	0.25 ± 0.00^{b}

Values with different superscript letters on the same column are significantly different at $p < 0.05$.

growth at 20˚C, values which are 112% and 124% higher, respectively, than the lowest OD observed at this temperature (0.30 ± 0.00 for 5P). Similarly, isolates 10G1 and 3M reached maximum growth at 35˚C (1.23 ± 0.04) and 40˚C (1.20 ± 0.03), demonstrating the high thermotolerance of these strains. At 15˚C, growth varied significantly ($p < 0.05$), with the 1M isolate showing the highest OD (0.85 ± 0.04), more than five times that of the 2M isolate (0.15 ± 0.00). Even at the stressful temperature of 45˚C, the 5P isolate maintained relatively high growth (0.75 ± 0.02), which was more than three times higher than the lowest value recorded at this temperature (0.21 ± 0.00 for 1M). These substantial differences in growth responses illustrate the high thermal adaptability between isolates and suggest significant physiological variation probably shaped by environmental pressures.

3.6. Distribution of the *Rhizobium* sp. Isolates According to Abiotic Factors (pH, Salinity, and Temperature)

A synthesis was carried out to identify isolates that simultaneously tolerate extreme pH, salinity, and temperature conditions, and the results are depicted. The isolates were classified into two groups according to these criteria. The first group includes growth at pH 2, NaCl 12%, and a temperature of 45˚C. The isolates belonging to this group were from TGX 2007 11 F, TGX 2001 12 F, SC Sentinel, Pan

1, Songda, Maksoy 2 N Pan 237, Pan 2, TGX 1988 18 F, S1180/15/54, TGX 1835-10 E, and Maksoy 1N. This means these varieties can be cultivated in highly acidic soils with high salinity and under high environmental temperatures. The second group includes growth at pH 2.5, NaCl 10%, and a temperature of 40˚C. It contains isolates from TGX 2007 11 F, TGX 2001 12 F, TGX 2010 3F, TGX 1991 22F, SC Sentinel, Pan 1, Pan 3, Songda, SC Signal, Maksoy 2 N, Pan 237, TGX 1989 60 F, TGX 1988 18 F, S1180/15/54, TGX 1835-10 E, and Maksoy 1N. These varieties can also be cultivated in hard environmental conditions. However, the isolates able to grow at high salinities (NaCl 12%) and low temperatures (between 15 and 20˚C) were from soybean varieties TGX 2007 11 F, Pan 1, Songda, SC Signal, Maksoy 2 N, Pan 237, Pan 2, TGX 1988 18 F, and TGX 1835-10 E.

3.7. Physicochemical Activities of the 73 *Rhizobium* sp. Isolates

Some physicochemical activities of the isolates, including antioxidant activity and inhibition of lipid peroxidation, were assessed in this study. Before that, the protein content of the isolates was determined. The presence of intracellular proteins in *Rhizobium* sp. induces the expression of nodulin genes that trigger the differentiation of cortical cells responsible for nodule formation, which are connected to the plant's conducting vessels, thus ensuring the system's energy supply. The protein content of the rhizobia cell-free supernatants is presented in Table 5. The isolates cell free supernatants showing the highest total protein concentrations were 10G2 (135.33 ± 5.65 µg/mL), 3M''' (122.22 ± 13.19 µg/mL), 5G (102.33 ± 19.01 µg/mL), 13M2 (110.88 ± 10.68 µg/mL), 15M1 (112.44 ± 18.54 µg/mL), 15M3 (109.88 ± 11.78 µg/mL), and 16G (106.33 ± 20.89 µg/mL). Lower protein concentrations were observed in isolates 1G (73.00 ± 2.67 µg/mL), 2M' (80.77 ± 0.47 µg/mL), 4M3 (82.44 ± 4.39 µg/mL), 5M2 (77.44 ± 6.12 µg/mL), 6G4 (82.55 ± 3.29 µg/mL), 12G3 (89.33 ± 0.62 µg/mL), and 13M (82.11 ± 6.44 µg/mL).

As different mechanisms may be involved in the antioxidant activity of the isolates, 3 methods were used: FRAP, TAC, and catalase. In the case of FRAP, the reducing power of ferrous ions varied according to the isolate. Regarding FRAP, isolate 7M from Pan 3 showed the highest ferrous ions reducing power (298.46 ± 0.00 µg AAE/mL). It was followed by isolates 15M2 (260.0 ± 0.00 µg AAE/mL) from S1180/15/54, 8P (225.76 ± 5.98 µg AAE/mL) from Songda, 6G (219.23 ± 17.94 0 µg AAE/mL) from Pan 1, and 4M3 (205.57 ± 17.13 0 µg AAE/mL) from TGX 1991 22F. Weak ferrous ions reducing power were observed with some isolates, including 11G3 (75.0 ± 2.17 µg AAE/mL) from Pan 237, followed by 6G3 (70.19 ± 2.44 µg AAE/mL) from Pan 1, 16M2 (69.23 ± 6.52 µg AAE/mL) from TGX 2001-10 DM, 8M'' (64, 61 ± 5.43 µg AAE/mL) from Songda, 1P (50.76 ± 1.63 µg AAE/mL) from TGX 2007 11 F, isolate 2G (46.92 ± 13.05 µg AAE/mL) from TGX 2001 12 F, 5M2 (43.65 ± 2.99 µg AAE/mL) from SC Sentinel, 15M4 (33.84 ± 4.35 µg AAE/mL) from S1180/15/54, and 14P2 from TGX 1988 18 F (14.61 ± 8.70 µg AAE/mL).

Concerning the total antioxidant capacity, the highest activities were obtained with isolates 4M1 (1335.93 ± 10.84 μg AAE/mL), 6G2' (1307.6 ± 9.42 μg AAE/mL), 10G2 (1194.6 ± 2.35 μg AAE/mL), 10G (1051.0 ± 9.33 μg AAE/mL), 7M (1033.93 ± 33.46 μg AAE/mL), and 5M (1033.93 ± 33.46 μg AAE/mL). These highly active isolates were from soybean varieties TGX 1991 22F (4M1), SC Sentinel (5M), Pan 1 (6G2'), Pan 3 (7M), and Maksoy 2N (10G2). Another antioxidant mechanism assessed in this study is through the production of catalase, an enzyme that inhibits the oxidation process by degrading the ROS called hydrogen peroxide. The results obtained showed that the catalase activity varies from one isolate to another. The highest catalase activity (0.07 ± 0.00) was recorded with isolates 1, 2G, 5P', and 13M, while the lowest ones (0.02 ± 0.00) were observed with isolate 10G2.

MDA is a stress marker in plants, and high MDA levels cause oxidative damage in plants, inhibiting their growth. In this study, the ability of isolates to inhibit MDA production was assessed. Generally, MDA values varied significantly ($p < 0.05$) from one isolate to another, depending on the soybean variety (Table 5). The isolates endowed with high inhibition of lipid peroxidation (low MDA values) were 4M1 (1.66 ± 0.11 μmoL/L) from TGX 1991 22F; 5P' (1.13 ± 0.13 μmoL/mL) from SC Sentinel; 8G (1.34 ± 0.12 μmoL/L) from Songda; 9P (1.70 ± 0.12 μmoL/L) from SC Signal; 10G1 (1.46 ± 0.01 μmoL/L) from Maksoy 2N; 11G3 (1.14 ± 0.05 μmoL/L) from Pan 237; 12G3 (1.42 ± 0.01 μmoL/L) from TGX 1989 60 F; 13M (51.48 ± 0.02 μmoL/L), and 14P4 (1.40 ± 0.04 μmoL/L) from TGX 1988 18 F. The low inhibition of lipid peroxidation (high MDA values) was recorded with isolates 16G (3.25 ± 2.07 μmoL/L) from TGX 2001-10 DM; 15M1 (2.74 ± 1.08 μmoL/L), 15M2 (2.85 ± 1.07 μmoL/L), and 15M3 (2.71 ± 0.69 μmoL/L) from S1180/15/54.

Generally, the isolates with the highest values of MDA, TAC, FRAP, protein, and catalase were from soybean varieties TGX 2007 11 F, TGX 2001 12 F, TGX 2010 3F, TGX 1991 22F, SC Sentinel, Pan 1, Pan 3, Songda, Maksoy 2 N, Pan 2, S1180/15/54, TGX 2001-10 DM, and TGX 1835-10 E.

Table 5. Protein content, antioxidant activity, and malondialdehyde content of 73 *Rhizobium* sp. isolates

Isolates	MDA (μmoL/L)	TAC (μg AAE/mL)	FRAP (μg AAE/mL)	Proteins (μg/mL)	Catalase (μmol/mL/g prot)
1	1.82 ± 0.20^{abcdefghijk}	640.26 ± 19.79^{abc}	85.19 ± 2.44^{efghij}	85.0 ± 2.67^{abcdefghi}	0.07 ± 0.00^{ab}
1G	1.37 ± 0.06^{abc}	396.6 ± 8.95^{opqrs}	117.5 ± 1.35^{lmnopqrst}	73.0 ± 2.67^{a}	0.05 ± 0.00^{mnopqrst}
1G'	1.49 ± 0.13^{abcdef}	247.26 ± 15.55^{ghijkl}	131.15 ± 7.07^{abcdefg}	99.11 ± 2.51^{ijklmnopqrstu}	0.05 ± 0.00^{abcdefgh}
1G''	1.53 ± 0.05^{abcdefgh}	248.6 ± 7.07^{ghijkl}	105.0 ± 10.33^{ijklmnopq}	95.44 ± 5.49^{defghijklmno}	0.04 ± 0.00^{bcdefghi}
1M	1.84 ± 0.01^{abcdefghijk}	324.6 ± 6.12^{opqrs}	142.30 ± 5.98^{abcde}	88.33 ± 7.07^{bcdefghijk}	0.06 ± 0.00^{abcdefg}
1P	1.58 ± 0.05^{abcdefghi}	251.26 ± 10.84^{ghijkl}	50.76 ± 1.63^{bcd}	87.66 ± 9.27^{abcdefghijk}	0.06 ± 0.00^{abcdefg}
2	1.37 ± 0.00^{abc}	58.26 ± 0^{a}	162.5 ± 2.44^{abcdefg}	96.22 ± 3.77^{efghijklmnop}	0.03 ± 0.00^{abcd}
2G	1.32 ± 1.10^{abc}	323.6 ± 18.85^{klmnop}	46.92 ± 13.05^{bc}	89.22 ± 3.61^{bcdefghijk}	0.07 ± 0.00^{a}
2M	1.74 ± 0.07^{abcdefghijk}	585.13 ± 0.65^{abc}	190.19 ± 6.79 ^{ab}	76.33 ± 9.89^{ab}	0.05 ± 0.00^{klmnopqr}
2M'	1.35 ± 0.12^{abc}	592.6 ± 4.24^{abc}	97.69 ± 7.61^{hijklmn}	80.77 ± 0.47^{abcd}	0.06 ± 0.00^{abcdefghij}

Continued

2P'C'	1.64 ± 0.13^{abcdefghij}	385.6 ± 15.08^{opqrs}	136.15 ± 31.54^{abcde}	88.55 ± 2.04^{bcdefghijk}	0.06 ± 0.00^{abcdef}
3G	1.77 ± 0.01^{abcdefghijk}	339.6 ± 65.05^{lmnopq}	124.42 ± 6.25^{pqrstuv}	96.88 ± 0.31^{fghijklmnopqr}	0.06 ± 0.00^{abcdefgh}
3M'	1.52 ± 0.34^{abcdefg}	538.26 ± 32.99^{abc}	173.84 ± 11.96^{abcd}	94.11 ± 2.04^{defghijklmn}	0.05 ± 0.00^{pqrstuv}
3M'''	2.01 ± 0.02^{cdefghijklm}	521.53 ± 1.97^{abcd}	98.26 ± 13.87^{hijklmno}	122.22 ± 13.19^{wx}	0.04 ± 0.00^{bcdefgh}
4M1	1.66 ± 0.11^{abcdefghij}	1335.93 ± 10.84^{a}	86.92 ± 2.17^{fghij}	99.77 ± 5.02^{ijklmnopqrstu}	0.06 ± 0.00^{abcdefg}
4M3	1.86 ± 0.00^{bcdefghijk}	385.26 ± 268.22^{opqrs}	205.57 ± 17.13^{ab}	82.44 ± 4.39^{abcdef}	0.06 ± 0.00^{abcdefg}
5G	1.66 ± 0.27^{abcdefghij}	289.93 ± 16.49^{ijklmn}	130.76 ± 2.71^{abcdefg}	102.33 ± 19.01^{klmnopqrstuv}	0.05 ± 0.00^{jklmnop}
5M	1.55 ± 0.25^{abcdefgh}	1033.93 ± 33.46^{a}	151.53 ± 20.12^{abcdefg}	97.55 ± 1.88^{ghijklmnopqrs}	0.05 ± 0.01^{opqrstu}
5M''	1.48 ± 0.16^{abcde}	434.26 ± 30.16^{abcde}	137.88 ± 6.79^{abcde}	95.44 ± 7.07^{defghijklmno}	0.05 ± 0.00^{nopqrstu}
5M2	1.58 ± 0.06^{abcdefgh}	496.8 ± 10.55^{abcd}	43.65 ± 2.99^{bc}	77.44 ± 6.12^{abc}	0.05 ± 0.00^{klmnopqr}
5P	1.54 ± 0.01^{abcdefgh}	72.26 ± 10.37^{a}	179.42 ± 7.34^{abcd}	98.88 ± 0.62^{hijklmnopqrstu}	0.03 ± 0.00^{bcdefgh}
5P'	1.13 ± 0.13^{a}	242.93 ± 1.88^{ghijk}	125.0 ± 9.24^{pqrstuv}	85.33 ± 5.97^{abcdefghi}	0.07 ± 0.00^{abc}
6G	1.58 ± 0.11^{abcdefgh}	450.6 ± 9.89^{abcde}	219.23 ± 17.94^{ab}	87.77 ± 2.82^{abcdefghijk}	0.05 ± 0.00^{qrstuvw}
6G'	1.83 ± 0.08^{abcdefghijk}	695.6 ± 0^{ab}	106.15 ± 13.59^{jklmnopq}	91.11 ± 4.39^{bcdefghijkl}	0.06 ± 0.00^{abcd}
6G2	2.41 ± 0.01^{klmn}	275.26 ± 31.58^{ghijklm}	191.15 ± 47.86^{ab}	100.55 ± 10.21^{jklmnopqrstu}	0.06 ± 0.00^{abcdefgh}
6G2'	1.53 ± 0.08^{abcdefgh}	1307.6 ± 9.42^{a}	144.80 ± 17.13^{abcde}	88.55 ± 4.87^{bcdefghijk}	0.05 ± 0.00^{lmnopqrs}
6G3	1.95 ± 0.09^{cdefghijkl}	863.6 ± 36.76^{ab}	70.19 ± 2.44^{def}	98.66 ± 6.59^{hijklmnopqrstu}	0.05 ± 0.00^{abcdefghi}
6G4	1.62 ± 0.04^{abcdefghi}	227.93 ± 81.55^{ghij}	121.34 ± 13.87^{opqrstu}	82.55 ± 3.29^{abcdef}	0.06 ± 0.00^{abcde}
7M	1.74 ± 0.21^{abcdefghijk}	1033.93 ± 33.46^{a}	298.46 ± 0^{a}	92.55 ± 9.89^{defghijklmn}	0.05 ± 0.00^{pqrstu}
7M'	1.99 ± 0.05^{cdefghijklm}	357.93 ± 22.15^{mnopqr}	167.30 ± 14.14^{abcdef}	92.11 ± 5.81^{cdefghijklmn}	0.06 ± 0.00^{abcdefg}
8G	1.34 ± 0.12^{abc}	376.26 ± 21.68^{nopqrs}	96.34 ± 8.43^{ghijklm}	95.11 ± 2.51^{defghijklmno}	0.06 ± 0.00^{abcdefghi}
8M'	1.43 ± 0.19^{abcd}	844.6 ± 21.21^{abc}	147.11 ± 9.51^{abcde}	96.55 ± 6.44^{fghijklmnop}	0.05 ± 0.00^{opqrstu}
8M''	1.60 ± 0.09^{abcdefghi}	359.6 ± 15.05^{mnopqrs}	64.61 ± 5.43^{cde}	111.77 ± 7.22^{rstuvw}	0.03 ± 0.00^{abcd}
8P	1.51 ± 0.04^{abcdefg}	676.93 ± 13.19^{abcd}	225.76 ± 5.98 ^{ab}	97.88 ± 0.47^{ghijklmnopqrst}	0.06 ± 0.00^{abcdefghi}
9G'	1.83 ± 0.07^{abcdefghijk}	690.93 ± 28.28 ^{ab}	88.46 ± 15.23^{fghij}	94.77 ± 0.15^{defghijklmn}	0.06 ± 0.00^{abcdefghij}
9P	1.70 ± 0.12^{abcdefghijk}	933.6 ± 57.51 ^{abc}	124.03 ± 10.06^{pqrstuv}	88.55 ± 5.81^{bcdefghijk}	0.06 ± 0.00^{abcdefghi}
10G	1.63 ± 0.19^{abcdefghij}	1051.0 ± 9.33^{a}	175.0 ± 16.86^{abcd}	93.88 ± 1.41^{defghijklmn}	0.06 ± 0.00^{abcdefgh}
10G1	1.46 ± 0.01^{abcde}	218.93 ± 158.32^{efghij}	147.69 ± 13.59^{abcdef}	94.88 ± 1.57^{defghijklmn}	0.04 ± 0.00^{fghijklm}
10G2	1.68 ± 0.31^{abcdefghijk}	1194.6 ± 2.35^{a}	168.65 ± 1.90^{abcde}	135.33 ± 5.65^{x}	0.02 ± 0.00^{a}
11G1	1.42 ± 0.23^{abcd}	380.6 ± 10.84^{nopqrs}	110.0 ± 7.61^{klmnopqr}	96.88 ± 4.71^{fghijklmnopqr}	0.06 ± 0.00^{abcdefghij}
11G2	1.67 ± 0.29^{abcdefghij}	768.26 ± 1.88^{abc}	165.96 ± 12.23^{abcdefg}	90.33 ± 1.09^{bcdefghijk}	0.06 ± 0.00^{abcdefgh}
11G3	1.14 ± 0.05^{ab}	359.6 ± 20.74^{mnopqrs}	75.0 ± 2.17^{efg}	113.55 ± 5.34^{uvw}	0.03 ± 0.00^{ab}
11G4	1.52 ± 0.22^{abcdefg}	542.26 ± 20.74^{abc}	187.30 ± 6.52^{abc}	86.44 ± 5.97^{abcdefghij}	0.05 ± 0.00^{nopqrstu}

Continued

11G5	1.56 ± 0.04^{abcdefgh}	68.93 ± 0^{a}	183.84 ± 9.79^{abc}	91.33 ± 0.62^{cdefghijkl}	0.04 ± 0.00^{ghijklmn}
12G	1.79 ± 0.08^{abcdefghijk}	451.6 ± 0.94^{abcde}	173.65 ± 3.53^{abcd}	93.33 ± 0.31^{defghijklmn}	0.06 ± 0.00^{abcdefghij}
12G2	1.57 ± 0.07^{abcdefgh}	181.6 ± 5.65^{bcdefg}	159.03 ± 4.62^{abcdefg}	100.55 ± 4.87^{jklmnopqrstu}	0.03 ± 0.00^{bcdefg}
12G3	1.42 ± 0.01^{abcd}	963.6 ± 27.34^{abc}	90.0 ± 11.42^{fghijk}	89.33 ± 0.62^{bcdefghijk}	0.06 ± 0.00^{abcdefgh}
13M	2.14 ± 0.10^{defghijklmn}	891.93 ± 1.41^{abc}	114.80 ± 10.60^{klmnopqrs}	82.11 ± 6.44^{abcdef}	0.07 ± 0.00^{ab}
13M2	1.73 ± 0.01^{abcdefghijk}	206.6 ± 1.41^{defghi}	82.5 ± 1.35^{efgh}	110.88 ± 10.68^{pqrstuvw}	0.04 ± 0.00^{cdefghij}
13M5	1.48 ± 0.02^{abcde}	434.26 ± 40.54^{abcde}	166.92 ± 5.98^{abcdef}	91.0 ± 0.15^{bcdefghijkl}	0.04 ± 0.00^{efghijkl}
14P2	1.52 ± 0.23^{abcdefg}	909.26 ± 8.01^{ab}	14.61 ± 8.70^{a}	85.44 ± 8.95^{abcdefghi}	0.05 ± 0.00^{jklmnop}
14P3	1.74 ± 0.19^{abcdefghijk}	412.93 ± 21.68^{pqrst}	114.61 ± 5.43^{klmnopqr}	111.55 ± 1.25^{qrstuvw}	0.04 ± 0.00^{bcdefghi}
14P4	1.40 ± 0.04^{abc}	126.26 ± 27.34^{abcde}	136.73 ± 8.97^{abcd}	91.55 ± 6.59^{cdefghijklm}	0.04 ± 0.00^{ijklmno}
15G	1.91 ± 0.25^{cdefghijkl}	191.6 ± 30.16^{cdefgh}	116.92 ± 4.89^{lmnopqrst}	94.0 ± 0.94^{defghijklmn}	0.04 ± 0.00^{ghijklm}
15M1	2.74 ± 1.08^{no}	800.26 ± 4.71^{abc}	178.26 ± 0.81^{abcd}	112.44 ± 18.54^{stuvw}	0.03 ± 0.00^{abc}
15M2	2.85 ± 1.07^{no}	88.6 ± 4.24^{ab}	260.0 ± 0^{a}	105.77 ± 11.94^{lmnopqrstuv}	0.05 ± 0.00^{jklmnopq}
15M3	2.71 ± 0.69^{mno}	103.26 ± 38.18^{abc}	131.73 ± 1.35^{abcdef}	109.88 ± 11.78^{opqrstuvw}	0.05 ± 0.00^{jklmnop}
15M4	2.17 ± 0.06^{efghijklmn}	722.26 ± 7.54^{abc}	33.84 ± 4.35^{ab}	96.22 ± 5.65^{efghijklmnop}	0.04 ± 0.00^{bcdefgh}
16G	3.25 ± 2.07^{o}	892.26 ± 19.79^{abc}	132.69 ± 7.61^{abcdef}	106.33 ± 20.89^{mnopqrstuv}	0.03 ± 0.00^{bcdef}
16M	2.25 ± 0.03^{hijklmn}	435.26 ± 6.12^{abcd}	238.07 ± 2.17^{a}	93.77 ± 15.08^{defghijklmn}	0.04 ± 0.00^{fghijklm}
16M1	2.21 ± 0.52^{fghijklmn}	239.93 ± 31.58^{ghijk}	119.23 ± 1.08^{nopqrst}	107.0 ± 3.61^{nopqrstuv}	0.03 ± 0.00^{abc}
16M2	2.31 ± 0.00^{ijklmn}	51.93 ± 52.32^{a}	69.23 ± 6.52^{def}	83.33 ± 2.82^{abcdefg}	0.03 ± 0.00^{abcd}
16M3	2.35 ± 0.48^{jklmn}	131.26 ± 15.55^{abcdef}	104.23 ± 0^{ijklmnopq}	91.22 ± 12.09^{bcdefghijkl}	0.04 ± 0.00^{efghijkl}
17G	1.94 ± 0.49^{defghijkl}	368.26 ± 23.57^{mnopqrs}	85.38 ± 1.08^{efghij}	96.77 ± 19.09^{fghijklmnopq}	0.04 ± 0.00^{ghijklmn}
17G1	1.82 ± 0.22^{abcdefghijk}	686.26 ± 12.25^{abc}	172.11 ± 0.81^{abcde}	84.0 ± 0.62^{abcdefgh}	0.05 ± 0.00^{jklmnop}
17G2	1.98 ± 0.05^{cdefghijkl}	280.26 ± 31.11^{hijklm}	167.88 ± 0.27^{abcdef}	109.88 ± 4.55^{opqrstuvw}	0.03 ± 0.00^{abcde}
17G4	2.62 ± 0.26^{lmno}	855.26 ± 173.94^{abc}	118.65 ± 1.35^{mnopqrst}	94.55 ± 1.72^{defghijklmn}	0.04 ± 0.00^{hijklmn}
17M	2.23 ± 0.03^{ghijklmn}	306.6 ± 2.37^{jklmno}	102.5 ± 6.79^{hijklmnop}	102.33 ± 2.98^{klmnopqrstuv}	0.03 ± 0.00^{bcdefg}
17M2	1.85 ± 0.11^{abcdefghijk}	280.26 ± 16.72^{hijklm}	94.42 ± 5.71^{ghijkl}	116.22 ± 4.39^{vw}	0.03 ± 0.00^{abc}
18G	1.53 ± 0.67^{abcdefgh}	223.93 ± 12.72^{fghij}	134.42 ± 0.81^{abcdef}	81.33 ± 2.82^{abcde}	0.06 ± 0.00^{abcdefgh}
18M	1.84 ± 0.15^{abcdefghijk}	1023.93 ± 9.89^{ab}	141.15 ± 0^{abcde}	112.77 ± 3.61^{tuvw}	0.03 ± 0.00^{abc}
18M2	1.60 ± 0.6^{abcdefghi}	116.93 ± 6.59^{abcd}	113.07 ± 2.17^{klmnopqr}	96.66 ± 15.08^{fghijklmnopq}	0.04 ± 0.00^{defghijk}
Control without isolate	1.4 ± 0.09^{abc}	453.93 ± 8.01^{abcd}	84.42 ± 5.71^{efghi}	91.11 ± 0.94^{bcdefghijkl}	0.06 ± 0.00^{abcdefg}

TAC = total antioxidant capacity; FRAP = ferrous reducing antioxidant power; MDA = malondialdehyde; Values with different superscript letters on the same column are significantly different at $p < 0.05$.

3.8. Principal Component Analysis

Principal component analysis (PCA) was used to explore associations between 73 *Rhizobium* sp. isolates, abiotic stress factors, protein content, and physicochemical activities. **Figure 2** illustrates the distribution of variables along the F1 × F2 axis, revealing four distinct groups with shared phenotypic and biochemical traits. Group 1 comprised 24 isolates, characterized by catalase activity and growth under conditions of moderate salinity (2% NaCl) and mesophilic temperatures (20-35˚C). Although catalase activity indicates a potential response to oxidative stress, the observed lack of tolerance to more extreme conditions, such as elevated salinity or temperature, suggests a limited overall adaptability to abiotic stress. These isolates can thrive in moderately stressed environments, but may be less competitive in more difficult soils. Group 2, located opposite group 1 on the PCA graph, included 15 isolates able to grow under high acid (pH 2 - 6.5) and salinity (up to 10% NaCl) conditions. This combination of acid and salt tolerance indicates robust adaptation mechanisms, probably involving efficient osmotic and pH homeostasis. These isolates are promising candidates for the development of bioinoculants in degraded acidic and saline soils, such as those found in various agroecological zones of Cameroon and sub-Saharan Africa. Group 3 consisted of 10 psychrotolerant isolates, able to grow at 15˚C and under stress conditions such

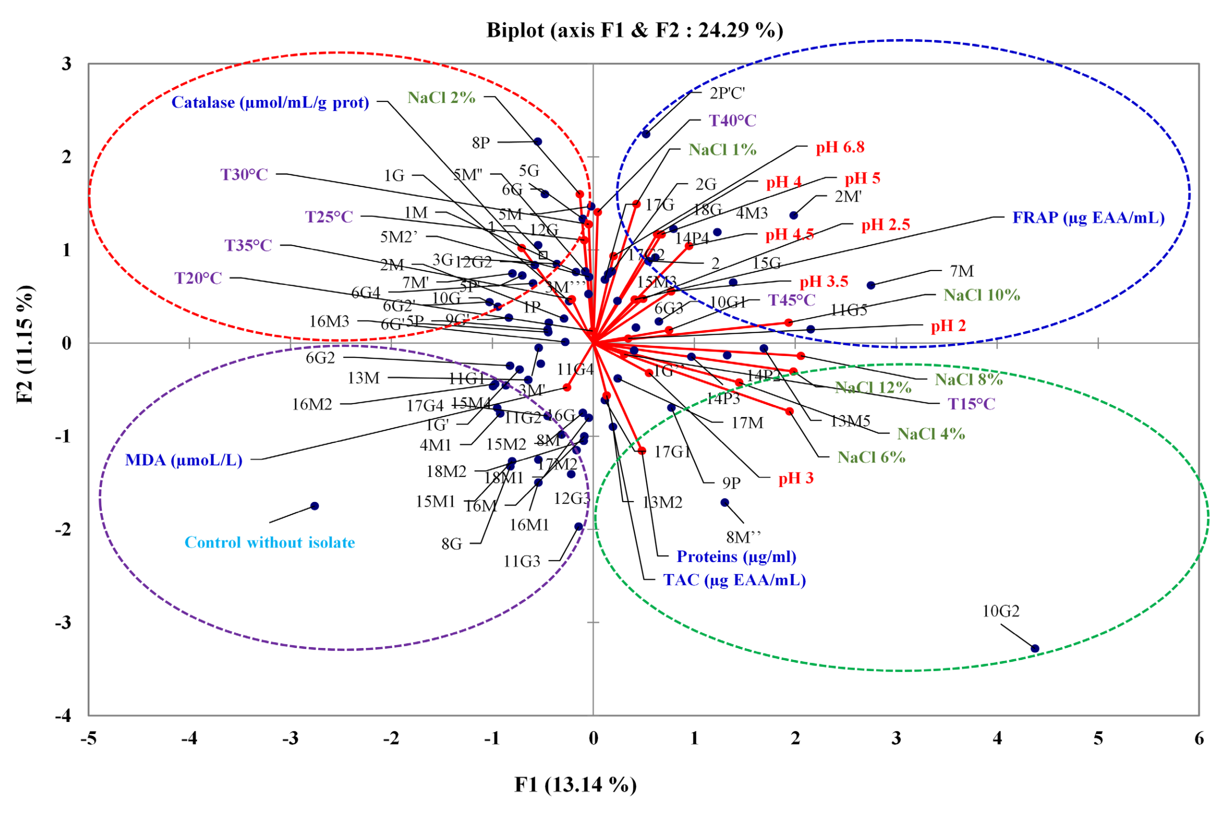

Figure 2. Principal component analysis showing the distribution of the 73 *Rhizobium sp.* isolates, the resistance of these isolates to abiotic parameters (pH, salinity, temperature), and their physicochemical activities on the F1 × F2 axis system.

as low pH (pH 3) and high salinity (12% NaCl). They also displayed high protein production and high total antioxidant activity. The clustering of these features suggests a potential synergistic response to abiotic stress, where protein biosynthesis may contribute to boosting antioxidant defenses. This group could harbor cold-adapted, stress-responsive enzymes or protective proteins, which would be useful for crops grown at high altitudes or in temperate tropical regions. Group 4 was defined by the presence of malondialdehyde (MDA), a marker of lipid peroxidation, and comprised 24 isolates. The association of these isolates with high levels of MDA suggests their involvement in oxidative stress responses, potentially linked to lipid membrane remodeling or reactive oxygen species scavenging. These isolates could enhance stress-induced cellular damage in host plants.

4. Discussion

Cameroonian soils are known for their acidic nature, which hampers the cultivation and productivity of several crops, including soybeans [9]. Amongst strategies for that issue, *Rhizobium* sp. appears as a good alternative. The microorganisms that can tolerate hard environmental conditions act in symbiosis with plants and help these latter to resist changes in abiotic parameters and the associated stress. In this context, the present study was designed to isolate and evaluate bacterial strains potentially belonging to the *Rhizobium* genus, capable of withstanding various abiotic stresses and endowed with physico-chemical activities favorable for plant growth under unfavorable conditions. The bacterial isolates were obtained from soybean root nodules. A total of 73 presumptive *Rhizobium* sp. isolates were obtained from nodules of 18 varieties of soybean farmed in acidic soil in Cameroon.

Acidity was the first abiotic parameter for which the tolerance of isolates was screened. Indeed, acidity affects nodulation during symbiosis through the excretion of isoflavonoids from soybean roots [34]. It also alters cell membrane permeability and damages roots, leading to reduced water and nutrient uptake, and jeopardizing the survival of soil micro-organisms [35]. According to Ferguson *et al.* [36], soil acidity alone is responsible for significant losses in the global production of legumes. Hence, it appears interesting to identify *Rhizobium* sp., which can resist to acidic conditions. In this study, the growth abilities of *Rhizobium* sp. isolates showed significant resilience across the pH range from 2 to 4. Some isolates showed a good ability to grow under a pH lower than 4. The most interesting acid-tolerant isolates were 14P3, 2, 17G2, 16G, 15M3, and 7M. This resistance to low pH can be explained by the ability of these isolates to produce extracellular polysaccharides or organic compounds in the cells. A similar observation was noticed in Argentina by Muglia *et al.* [37]. The results of this study suggest the capacity of isolates 14P3, 2, 17G2, 16G, 15M3, and 7M to establish a symbiotic relationship with soybean even in an acidic environment, and boost their productivity. It is in direct line with findings of Nkot *et al.* [38], who demonstrated that acid-tolerant strains of rhizobia can successfully establish under acid environmental conditions, symbiosis with the legume *Vigna unguiculata*, and improve its nitrogen uptake and productivity.

Besides acidity, temperature was an important abiotic parameter assessed in this study. The rhizobia isolates displayed various growth rates at the different temperatures tested. This can be related to the fact that rhizobia are ubiquitous bacteria that thrive in subarctic, temperate, and tropical regions and therefore respond to different environmental conditions [39]. However, most of the isolates showed optimum growth at temperatures between 25˚C and 30˚C. 52% of isolates were unable to grow at 15˚C, and 62% at 40˚C and more. A few proportions of isolates displayed optimal growth at low temperatures, with 1M and 4 M1 showing the optimal growth at 15˚C and 20˚C, respectively. This result shows that they can be suitable for soils of temperate regions. However, the negative effects of low temperature such a reduction of rhizobia competitiveness, a reduction of the synthesis and secretion of the Nod factor, inhibition of the symbiotic process, and restriction of nodule development [40]. The limited growth of isolates at 40˚C and above may be explained by an alteration in key processes of rhizobia symbiosis, including bacterial adhesion, nodule initiation, and nitrogenase activity. Recent studies show that high temperatures disrupt these steps, reducing the efficiency of nitrogen fixation [41]. High temperature depletes photosynthetic activity, decreases absorbing hair formation, reduces nodulation sites, and alters bacterial adhesion to absorbing hairs [42] [43]. However, there are some isolates (15M3, 10G1, 9P, 5P, 5P’, 2, and 1G) for which optimum growth was at 45˚C. The growth at high temperatures recorded with these isolates can arise from their ability to synthesize heat shock proteins [44]. It can also be explained by the origin of the isolates. Rhizobia isolated from subtropical regions like the sampling site of this study are known to be resistant to high temperatures [45]. According to Zhang *et al.* [46], isolates that are tolerant to stress, particularly high temperatures, can maintain efficient nitrogen fixation under unfavorable abiotic conditions. That resilience also promotes a constant supply of nitrogen by rhizobia, thus compensating for the low natural fertility of these soils [47]. Hence, there is a need for further investigations on the effect of inoculating these thermotolerant isolates (15M3, 10G1, 9P, 5P, 5P', 2, and 1G) on soybean seeds.

The last abiotic parameter assessed in this study was salinity. Salt stress impairs crop growth and yield by reducing photosynthetic activity through reduced gas exchange, altering morphological development, disrupting membrane functions, and affecting antioxidant activities [48]. Hence, identifying rhizobia endowed with resistance to salinity can be beneficial for protecting leguminous crops through the regulation of oxidative stress, balancing of phytohormone levels, and secretion of osmolytes [49] [50]. In this study, the isolates showed variable growth in the presence of different NaCl concentrations. This variation could be attributed to the difference in metabolic activities leading to the production of compounds associated with salinity resistance as reported by Mhadhbi *et al.* [51] and Del Cerro *et al.* [52]. That property is very important for plant-rhizobia symbiosis. In fact, during salinity stress, the plant can survive if the rhizobia with which they are in symbiosis persist in soils and keep their ability to colonize, infect, produce nodulation factors, and fix nitrogen. At the high NaCl concentration of 12%, the optimum growth was recorded with isolate 10G2. The tolerance of isolate 10G2 to high NaCl concentration can be explained by its ability to produce and accumulate osmoprotectants, such as proline, thus helping to limit the deleterious effects of osmotic stress [50] [53]. In addition, some *Rhizobium* sp. strains have demonstrated their ability to mitigate the ionic toxicity of NaCl through the production of exopolysaccharides or the regulation of ionic homeostasis. This dual action can promote the establishment and functioning of the soybean-*Rhizobium* symbiosis under salinity conditions, by promoting the initiation of nodulation and sustaining nitrogen fixation activity. The observed resistance of isolate 10G2 to high NaCl concentrations can be helpful for the soybean-*Rhizobium* symbiosis. It helps the plant during nodulation initiation and improves its nitrogen-fixing capacity under high salinity stress. Khan *et al.* [54] showed that inoculation of soybeans with halotolerant isolates improved the growth, biomass, and chlorophyll content of seeds farmed on a salty soil. These strains promote ionic homeostasis by increasing K^+ uptake and limiting Na^+ accumulation, while stimulating antioxidant activity. They also induce the expression of genes associated with salt tolerance, such as GmST1 and GmLAX3, contributing to improved physiological resilience. Furthermore, Shahid *et al.* [55] showed that some halotolerant strains contribute to the resilience of legumes by producing phytohormones that promote growth and nodulation, thus improving the ecological integration of plants in constrained environments while maintaining nitrogen fixation.

The production of proteinaceous compounds is amongst the resistance mechanisms of rhizobia to abiotic stress conditions. According to Boominathan and Doran [56], under edaphic stress, enzymes are secreted in rhizobia for their protection. In this study, the isolates have demonstrated the ability to grow and survive under extreme conditions of pH, temperature, and salinity.

The protein content of isolates was assessed and revealed significant variability between strains. Such variations are commonly observed among microbial isolates and may reflect differences in metabolic activity, stress tolerance mechanisms, or adaptation to environmental conditions. Up-regulation of genes encoding for proteins and lipopolysaccharides (LPS) in *Rhizobium* sp. exposed to a temperature of 43˚C was reported by Nandal *et al.* [57]. Studies by Gomes *et al.* [58] in Brazil showed that *Rhizobium tropici* strain PRF81, when exposed to heat stress, increased the synthesis of proteins such as bacterioferritin and thioredoxin, associated with oxidative stress responses. The highest protein values, recorded in this study with isolate 10G2 (135.33 ± 5.65 µg/mL), followed by 3M''' (122.22 ± 13.19 µg/mL) and 5G (102.33 ± 19.01 µg/mL), suggest that these isolates might be suitable for application in soybean production under stress conditions.

The proteins produced in response to stress conditions generally have different roles. Some are involved in the constitution of cell structure, while others directly act on the compounds generated by stress conditions. The main compounds generated by stress conditions are free radicals and reactive oxygen species (ROS) that affect cell function, leading to oxidative damage and ultimate plant death [59]. One of the proteins involved in the antioxidant mechanism is catalase. That enzyme plays an essential role in trapping ROS and thus preventing oxidative damage [60]. The catalase activity of the different isolates was assessed in this study. All the isolates were positive with activity ranging from 1033.93 ± 33.46 to 1335.93 ± 10.84 µg AAE/mL. The variation in catalase activity can be explained by the difference in the metabolic mechanism of synthesis of that enzyme, which occurs in the peroxisomes. Indeed, the synthesis of catalase in the peroxisome is induced by the substrate, and varies according to the microorganism, the type of cell, and the environmental conditions [61]. The catalase activity recorded in this study with rhizobia isolates suggests that they can be used in soybean production to maintain the plant's pro-oxidant-antioxidant balance and ensure good functioning of the symbiotic relationship under stressful conditions. Kots *et al.* [14] highlighted that catalase activity is linked to the symbiotic nitrogen uptake activity in legume root nodules and depends on the efficiency of the *Rhizobium*-legume symbiosis.

Besides catalase activity, other antioxidant mechanisms of the rhizobia isolates were assessed through the ferric reducing power and total antioxidant capacity. The results obtained showed that all isolates were positive for TAC and FRAP activities, with values that vary significantly from one isolate to another. The highest TAC values were obtained with 4M1 (1335.93 ± 10.84 µg AAE/mL), 6G2' (1307.6 ± 9.42 µg AAE/mL), 10G2 (1194.6 ± 2.35 µg AAE/mL), 10G (1051.0 ± 9.33 µg AAE/mL), 7M, and 5M (1033.93 ± 33.46 µg AAE/mL). A similar trend was observed with the FRAP activity of the isolates. This means that the isolates can protect plants against stress due to an imbalance in favor of free radicals and pro-oxidants. Hence, they might be suitable for Cameroonian soils, for which soybean plants are exposed to several abiotic stress factors.

Lipids play an important role in signal transduction in plant response to abiotic stress [62]. When plants are stressed, the free radicals and ROS generated can affect membrane properties by oxidizing membrane lipids. In response to lipid oxidation, plants increase their content of some compounds, such as MDA. MDA represents the main marker used to assess lipid oxidation [63]. The accumulation of MDA in cells after exposure to stress was highlighted by Thounaojim *et al.* [64]. In the present study, the MDA levels of the isolates were measured. Isolates 16G (3.25 ± 2.07 µmoL/L), 15M1 (2.74 ± 1.08 µmoL/L), 15M2 (2.85 ± 1.07 µmoL/L) and 15M3 (2.71 ± 0.69 µmoL/L) recorded the highest MDA levels. These rhizobia isolates may exhibit a relatively low antioxidant activity as suggested by their high levels of MDA, which are indicative of oxidative stress and reduced antioxidant capacity [65]. While the other isolates scored significantly reduced MDA contents. The significant variation in the MDA contents can be explained by the variability in the production of antioxidant metabolites by the isolates. That property is very important for soybeans as rhizobia isolates can regulate membrane lipid peroxidation and thus, protect plants from severe oxidative damage [66].

Considering the overall growth and biological activities, Santos *et al.* [67] in their studies in Brazil noticed that under a high salinity environment, halotolerant strains of *Bradyrhizobium* stimulate *Vigna unguiculata* resistance to stress through the production of antioxidants, including catalase. Manassila *et al.* [68] highlighted that the acid tolerance ability of *Bradyrhizobium* sp. DASA01007, isolated from acidic soils in Thailand, might promote an efficient symbiosis in acidic environments. In the present study, we identified rhizobia strains endowed with acid tolerance, salt tolerance, high synthesis of proteins, high ability to inhibit lipid oxidation, and noticeable antioxidant activities. Thus, suggesting their unique ability and potential to promote through symbiosis, the growth of legumes, particularly soybean, under stress conditions.

The results obtained in this study open up prospects for identifying the most promising isolates and assessing their effects on agronomic performance parameters of soybean. By identifying the most efficient strains for the production of commercial inoculants, we will not only improve the efficiency of fermentation processes but also select strains better suited to various biotechnological applications, such as biomass and biofertilizer production. In agronomic terms, this research contributes to enhancing soil fertility by providing crucial information on which strains are most effective under specific conditions. This in-depth understanding can guide the choice of the best options for crops while supporting sustainable agricultural practices. By promoting the use of adapted strains, we could also reduce dependence on chemical fertilizers, thus promoting more environmentally friendly agriculture.

5. Conclusion

In this study, 73 presumptive *Rhizobium* sp. isolates were obtained from 18 varieties of soybeans. The majority of these isolates demonstrated the ability to grow and survive in extreme conditions of acidity, salinity, and temperature. The adaptation was adapted differently from one isolate to another according to the various environmental stresses encountered. The isolates displayed antioxidant activities through different mechanisms, thanks to the ability to produce proteins endowed with antioxidant activity, like catalase. They also demonstrated the ability to inhibit lipid peroxidation through the inhibition of MDA production. The combination of the antioxidant potential of *Rhizobium* sp. isolates and resistance to abiotic factors suggests that they might be suitable as inocula for large-scale production of soybeans in acidic soils. This study enabled morphological and physiological characterization of *Rhizobium* sp. isolates. For that, there is a need for further studies on the exploration of their taxonomic diversity using molecular tools. The evaluation of *Rhizobium* strains is of crucial importance for their adaptation to specific environments. By examining the behavior of these strains under a variety of conditions (such as soil types and climates), we can fine-tune inoculation strategies to suit local realities. Furthermore, selecting strains capable of withstanding extreme conditions, such as drought and salinity, can significantly boost crop productivity in times of environmental stress. Finally, exploring the interactions between different *Rhizobium* strains and other microorganisms opens the way to strengthening synergies, thereby improving the efficiency of cultivation practices. This integrative approach could therefore play a key role in optimizing cropping systems and agricultural sustainability.

Data Availability

Data available on request from the authors.

Authors' Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by LM, HM, EL, OB, FL, GM, and AD. The first draft of the manuscript was written by LM and all authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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