
SHORT COMMUNICATION

Salinity tolerance of endophytic fungi associated with Pokkali rice from coastal saline ecosystems of Kerala

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Received : 01.06.2026

Accepted : 26.07.2026

Published : 28.09.2026

The Pokkali rice ecosystem situated in Kerala, India, is a distinctive saline agroecosystem, containing diverse microorganisms with unique stress-adapted characteristics. The evaluation of endophytic fungi was based on rice samples collected from three distinct locations (Kalamassery, Njarakkal, and Palluruthy) within the Pokkali rice ecosystem located in the Ernakulum District of Kerala, India. The assessment of salinity tolerance of endophytic fungi was achieved by growing the fungi on potato dextrose agar media (PDA) that was amended salt to attain with electrical conductivity (EC) measured at 4, 8, and 12 dS m⁻¹ levels, and the results were expressed as the percent retention of the growth (GR) of the endophytic fungi relative to the control. The endophytic fungi isolated from samples demonstrated a high degree of variability in their salt-tolerance capabilities. At the 12 dS m⁻¹ salinity level, the GR of all endophytic fungi tested ranged from 33.33% (KLEF-14) to 107.23% (KLEF-18). The endophytic fungus KLEF-18 had the highest GR value at 107.23% when compared to the no-added salt control, followed by KLEF-6 which had a GR value of 102.67%. Also, KLEF-8, NLEF-3, and NLEF-2 all had GR values of 102% or greater and were statistically comparable to each other. Many of the endophytic fungi isolated demonstrated a GR value close to or greater than 100% when grown on PDA with increased salinity, indicating these fungi are well adapted to salt-stressed conditions. Comparatively, KLEF-14, KLEF-15, KLEF-2, and NREF-1 exhibited low GR values and therefore these endophytic fungi have been identified as salt-sensitive endophytes. Five endophytic fungi (KLEF-18, KLEF-6, KLEF-8, NLEF-3 and KLEF-9) evaluated have demonstrated their potential as salt-tolerant endophytes for future evaluation for use in managing salinity-induced stress and for sustainable crop production.

Keywords : Endophytic fungi, growth retention, Pokkali agroecosystem, Pokkali rice, salinity tolerance, saline ecosystem,

INTRODUCTION

Pokkali is a unique traditional rice landrace cultivated in the coastal saline regions of Kerala, India; characterized by its tolerance to salinity, flooding and multiple environmental stresses. The Pokkali rice ecosystem is therefore an example of a naturally evolved agroecological system, consisting of natural populations of microorganisms found in an environment experiencing fluctuating levels of salinity. The lasting effects of saline conditions on plants and

associated microorganisms have given rise to unique microbial communities that have evolved to be able to survive and function under conditions of extreme salt stress.

Endophytic fungi are microorganisms which colonise internal plant tissue without causing disease as well as establishing beneficial relationships with the host plant; they enhance plant growth and increase nutrient acquisition, provide enhanced tolerance to abiotic stress, and contribute to overall plant health through the production of various bioactive compounds. Of special interest are endophytic fungi that grow in stress-prone environments as they will often exhibit various adaptive traits that allow them to

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survive in these extreme stress conditions and may also confer some level of stress tolerance to the plants that they inhabit. Salinization of soils is a significant abiotic factor that is limiting agricultural production on a global basis and the impacts of salinization on agricultural output will further deteriorate due to the effects of climate change, seawater intrusion and unsustainable irrigation practices. As a result, identifying salt-tolerant microorganisms has received considerable attention as a potential eco-friendly approach to increase the productivity of crops grown in saline soils. Potentially, fungal endophytes that exhibit the ability to grow in saline conditions could provide advantages as candidates for producing microbial inoculants to improve crop adaptability to salt stress.

In consideration of particular ecological features existing within the Pokkali ecosystem, the objective of the current study was to isolate endophytic fungi associated with Pokkali rice and assess their tolerance to salinity using *in vitro* growth conditions. The isolated endophytes were screened for salinity tolerance by culturing each isolate on salinity-amended potato dextrose agar (PDA) with differing electrical conductivity levels and assessing growth of each isolate under salt stress to determine potential salt-tolerant endophytes for future agricultural applications.

MATERIALS AND METHODS

The salinity tolerance of endophytic fungi obtained from Pokkali rice was tested *in vitro*. Pokkali rice plants were collected from three padasekharams in the Ernakulam district of Kerala, India (Kalamassery, Njarakkal and Palluruthy) for endophytic fungi isolation, purification, and growth on potato dextrose agar (PDA) and evaluation of the salinity tolerance. The isolates were grown in PDA at three levels of salinity (4, 8, and 12 dS m⁻¹; EC) and PDA with no salt amendment served as the control. Mycelial discs (5 mm) taken from the actively-growing edge of the culture at 7 days were placed in the centre of the dish. The plates were incubated at 28 ± 2°C until the fungal growth achieved the outer edge of the control plate before measuring the diameter of the two perpendicular colony diameters and recording their average.

The percentage of growth retention of the isolates at each salinity level compared to the corresponding growth retention of the control was calculated to assess the ability of the isolates to tolerate salt stress.

RESULTS AND DISCUSSION

At the saline stress levels examined (4 EC, 8 EC, and 12 EC), the endophytic fungal isolates were found to have considerable variation in their ability to retain growth (Table 1). All of the isolates were labelled with an identification code of NRE (Njarakkal Root Endophytic Fungi), NSE (Njarakkal Stem Endophytic Fungi), NLE (Njarakkal Leaf Endophytic Fungi), KLE (Kalamassery Leaf Endophytic Fungi), PRE (Palluruthy Root Endophytic Fungi) and PLE (Palluruthy Leaf Endophytic Fungi), based on what part of the plant they were associated with and where they were obtained from. Most isolates had high retention values at 4.0 EC indicating that the low salinity levels were not limiting to the growth of the mycelium. At 8.0 EC, the trend continued with a high number of isolates having greater than 95% retention of original growth rates. The isolate labelled KLEF-6 had the greatest retention of growth at this salinity level (at 112.00%) followed by KLEF-18 (106.02%), NLEF-3 (104.65%), KLEF-9 (102.33%), KLEF-8 (101.16%), KLEF-7 (101.15%) and NSEF-1 (101.15%). From these data, it can be concluded moderate salinity levels did not inhibit the growth of these isolates and may have stimulated their growth.

At the highest salinity level (12 EC), growth retention varied between 33.33 and 107.23%. The highest growth retention was exhibited by KLEF-18 (107.23%) while the isolate KLEF-6 (102.67%) was not significantly different from this isolate but was far superior to all of the other isolates tested. The isolates KLEF-8 (102.33%) and NLEF-3 (102.33%) were also statistically similar and showed significant tolerance to salinity. KLEF-9 (101.16%) also had a high growth retention value with significant differences from the lower retention values shown by some of the isolates. The isolates KLEF-17 and KLEF-5 had 100% retention of the initial growth value and were statistically similar to one another in terms of their

Table 1: Growth retention (%) of endophytic fungal isolates from Pokkali rice under different salinity levels

Isolate	Growth retention (%) 4 EC	Growth retention (%) 8 EC	Growth retention (%) 12 EC
KLEF-1	98.86 ^f	96.59 ^j	86.36 ^t
KLEF-2	66.67 ^r	55.07 ^t	50.72 ^w
KLEF-3	100.00 ^d	100.00 ^g	98.89 ^g
KLEF-4	95.51 ¹	88.76 ^a	89.89 ^p
KLEF-5	100.00 ^d	100.00 ^g	100.00 ^f
KLEF-6	109.33 ^a	112.00 ⁿ	102.67 ^b
KLEF-7	100.00 ^d	101.15 ^f	98.85 ⁱ
KLEF-8	102.33 ^k	101.16 ^d	102.33 ^a
KLEF-9	96.51 ^k	102.33 ^d	101.16 ^a
KLEF-10	98.86 ^r	96.59 ^j	98.86 ^h
KLEF-11	96.67 ^j	96.67 ^j	90.00 ^o
KLEF-12	100.00 ^d	95.56 ¹	96.67 ^k
KLEF-13	72.60 [?]	95.89 ^k	89.04 [?]
KLEF-14	80.30 ⁿ	56.06 ^a	33.33 ^y
KLEF-15	65.88 ^a	62.35 ^t	48.24 ^t
KLEF-16	97.70 ⁱ	91.95 ⁿ	79.31 ^a
KLEF-17	100.00 ^d	100.00 ^g	100.00 ^t
KLEF-18	80.72 ⁿ	106.02 ^b	107.23 ⁿ
KSEF-1	96.67 ^j	97.78 ^h	96.67 ^k
NLEF-1	80.25 ^g	76.54 ^t	67.90 ^m
NLEF-2	57.47 ⁿ	54.02 ⁿ	51.00 ⁿ
NLEF-3	103.49 ^b	104.65 ⁿ	102.33 ⁿ
NREF-1	62.07 ^t	45.98 ^v	51.72 ^v
NSEF-1	98.85 ^g	101.15 ^f	95.40 ^m
PLEF-1	97.78 ^h	94.44 ^m	92.22 ⁿ
PLEF-2	100.00 ^d	96.67 ^j	96.67 ^k
PLEF-3	96.67 ^j	96.67 ⁱ	95.56 ¹
PLEF-4	98.89 ⁿ	97.78 ^h	97.78 ^j
PREF-1	82.35 ^m	74.12 [?]	69.41 ^t

Mean ($\pm$ SD) of three replications. Values followed by similar superscripts in a column are not significantly different at 5% level

overall growth rates. In contrast, KLEF-14 recorded the lowest growth retention (33.33%), followed by KLEF-15 (48.24%), KLEF-2 (50.72%) and NREF-1 (51.72%), indicating significant sensitivity to salinity stress.

It could be hypothesized that the high growth rates exhibited by many of the strains tested at high salt concentrations may be linked to the fact that the majority of the source of these strains

originated from a Pokkali ecosystem that is naturally subjected to a range of varying saline environments. Previous research has shown that endophytes found in salt-adapted plants can perform better than non-salt-adapted endophytes in terms of their tolerance to osmotic and ionic stress; therefore, they may assist the plant host in adapting to such adverse environmental conditions. Many comparative studies have demonstrated that endophytes from salt-adapted

plants, such as Pokkali rice, can improve the growth of plants subjected to salt stress through: osmotic adjustments; production of phytohormones; regulation of antioxidants and improved stress signaling (Chitnis *et al.* 2020; Sampangi-Ramaiah *et al.* 2020; Du *et al.* 2026).

The present screening identified KLEF-18, KLEF-6, KLEF-8, NLEF-3 and KLEF-9 as the most promising salt-tolerant fungal endophytes. The ability of these fungi to show 100% or more of their growth at 12 EC indicates a strong adaptation to a saline environment. These fungi can be targeted for future investigations regarding their capability to promote plant growth, develop stress tolerance and improve crop productivity when grown in saline soil.

DECLARATIONS

Conflict of Interest. Authors declare no conflict of interest.

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