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Comparative analysis of two predominant methods of rice sheath blight inoculation

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ABSTRACT

Sheath blight of rice is an important fungal disease caused by Rhizoctonia solani Kuhn. The two most commonly used rice sheath blight inoculation methods, agar block and colonized typha bit inoculation were compared on the basis of mass multiplication and inoculation. The results revealed that the agar block method took single day to form mycelia and 8 days to form sclerotia in the petriplate whereas, typha method took 2 days and 15 days for the same . The symptom expression on popular susceptible check, BPT-5204 took 4 days in former compared to seven days in later. The total time taken for mass multiplication was less in case of agar block method whereas; the inoculation was faster in case of typha method. Thus, the agar block method is appropriate for symptom expression and omic studies whereas, the typha method is well suited for large scale germplasm screening in field conditions.

Keywords **:** Agar block method, colonized typha bit inoculation method, mycelia, *Rhizoctania solani*, sclerotia, sheath blight

Rice is an important cereal crop belonging to the genus *Oryza* and the family Poaceae. The major cultivated forms of rice are *Oryza sativa* L. (Asian rice) and *Oryza glaberrima* L. (African rice). During the year 2020, the global rice production was estimated to be around 756 million tonnes, out of which nearly 90 per cent was contributed by Asia followed by 5 per cent from Africa (FAO, 2021). Among the Asian countries, China, India, Bangladesh and Indonesia are leading in terms of rice area and production (Statista, 2020-21).

The world's rice production has been affected by a number of factors such as shrinkage of arable lands, growing concerns over sustainable agriculture and most importantly, biotic and abiotic stresses. Among the biotic factors, the diseases are the key contributors which lead to an annual yield loss of up to 30 per cent (Skamnioti and Gurr, 2009).

Sheath blight (ShB) is a major fungal disease of rice and ranks next to the rice blast in terms of economic damage. In India, the yield loss due to rice ShB ranges from negligible to sixty nine per cent depending upon the disease severity, stage of the crop affected and the favorable ecological conditions (Sivalingam *et al*., 2006). The rice sheath blight was first reported from Japan and is known to be caused by *Rhizoctonia solani* Kuhn (Miyake, 1910). The symptoms include formation of water-soaked, oval to irregular shaped grayish colored lesions with brown margin on the leaf sheath which ultimately leads to stem lodging and improper grain filling (Wu *et al*., 2012).

The disease is mainly controlled by application of fungicides, however; these fungicides are not environmentally safe and have side effects on beneficial microorganisms. Hence, identification of resistant varieties by screening studies is the only alternative to manage the disease. The partial resistance based on quantitative trait loci (QTL) is mainly used for the crop improvement against this disease as no major ShB resistant gene has been identified till date. The majority of the identified ShB-QTLs have been linked to the plant morphological traits which have detrimental effects on the yield parameters. Additionally, the agronomic traits such as tall stature, late maturing habit and open canopy with few tillers are influenced by environmental factors and thus, pose difficulties in the detection of complete resistance in case of rice ShB (Li *et al*., 2022; Park *et al*., 2008). Hence, an inoculation method that minimizes the effect of environmental factors and results in measurable infection irrespective of the plant morphology will be most appropriate for evaluating the rice genotypes against ShB infection.

Several inoculation techniques have been tried in the past to identify the resistant entries against rice ShB such as colonized typha bit inoculation (Bhaktavatsalam *et al*., 1978), bamboo-toothpick inoculation (Zou *et al*., 2000), syringe inoculation (Wasano and Hirota, 1986), sclerotial inoculation (Singh *et al*., 2001), rice straw inoculation (Che *et al*., 2003), agar block method (Jia *et al*., 2007), toothpick inoculation (Prasad and Eizenga, 2008) and mycelial inoculation (Park *et al*., 2008). The

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techniques which are effective in one environmental condition may fail in another and the varieties with open growing habit may escape the infection during the screening. Among these techniques, colonized typha bit inoculation and agar block method are most commonly used and have been proved to be more efficient in evaluating the rice genotypes against ShB under artificial and natural conditions. Thus, in order to analyze the key difference between these two techniques and to find out which one is superior the current study was undertaken.

MATERIALS AND METHODS

The comparative inoculation studies were carried out in the greenhouse facility at Department of Biotechnology, University of Agricultural Sciences (UAS), Dharwad, Karnataka, India.The temperature was maintained at 28 °C for the day time and 23 °C for the night time for maximum disease incidence.

Sowing of plant material

The popular rice variety, BPT-5204, which has been already proven to be highly susceptible to rice ShB (Yadav *et al*., 2015) was utilized for this pot screening studies. The pots were filled with sterilized soil and good quality seeds of BPT-5204 variety were sown in two batches for the two methods of inoculation. The rice plants were inoculated against *R. solani* at the late tillering stage (45-50 days after sowing). The experiment was replicated five times in order to cross check the results.

Fungal inoculum.

The virulent isolate of *R. solani*, RS4, (GenBank Accession No MK213724) (Suryawanshi *et al*., 2019) maintained at Institute of Agri-Biotechnology, UAS, Dharwad was used for this comparative analysis.

Sheath blight inoculation using agar block method

For agar block inoculation method, the pure culture of RS4 was obtained by inoculating onto water agar. Later, the agar block from the outer edge of a two-days-old culture was transferred to a petriplate containing fresh potato dextrose agar (PDA) medium using cork borer and inoculation loop. The petriplates were incubated at room temperature for ten days and later used for inoculation purpose (Fig. 1A).

Each plant was individually inoculated with two agar blocks containing sclerotia along with mycelia at maximum tillering stage. The rice sheath was opened and 0.5 cm diameter agar block was placed into it with the help of forceps. The inoculated portion was covered with moist cotton and aluminium foil to avoid loss of moisture (Jia *et al*., 2007). Later, the plants were covered with polythene bags to maintain appropriate humidity and induce maximum disease (Fig. 2).When the typical lesions appeared after 4 days, the aluminum foil, moist cotton and polythene covers were removed.

Sheath blight inoculation using colonized typha bit inoculation method

The typha (*Typha angustata* L.) bit method of inoculation was conducted according to Bhaktavatsalam *et al*. (1978) with some modifications. For this, typha shoots were collected and cut into 4.0-5.0 cm bits with the help of sterilized knife. The bits were washed, soaked in distilled water for two hours and dried using tissue paper. The dried typha bits were transferred to a conical flask and autoclaved twice on two consecutive days. Later, the typha bits were inoculated with three days old actively growing culture of RS4 using cork borer and inoculation loop in sterile condition.The inoculated typha bits were incubated at room temperature for 12-15 days or till the time the typha bits were completely covered with fungal mat and sclerotia was formed over it (Fig. 1B). The fifteen days old typha bits with mycelia and sclerotia were placed in between the tillers for sheath blight disease inoculation (Fig. 3)

Observations

The observations such as days to form first mycelium and sclerotium, time taken by mycelia to completely cover the petriplate/conical flask walls were recorded during the mass multiplication phase and incubation period in terms of days from inoculation to the appearance to first visible symptom was observed during inoculation phase (Yeh and Bonman, 1986). Apart from that, the disease reaction of BPT-5204 against *R. solani* was measured in terms of relative lesion height percentage (RLH %) on 21st day post inoculation (dpi) for both the inoculation methods. The RLH % was calculated by the formula

$$\text{RLH\%} = \frac{\text{Height of ShB lesion on plants(cm)} \times 100}{\text{Height of Plant (cm)}}$$

The disease scoring in terms of RLH percentage was carried out using 0-9 grade scale of Standard Evaluation System (SES) (IRRI, 2002).

RESULTS AND DISCUSSION

The type of inoculum used and the technique employed for inoculation determines the efficiency of ShB screening studies (Park *et al*., 2008). In this study, the comparative analysis of two rice sheath blight inoculation methods was performed at culture preparation and inoculation phase. During culture preparation, it was observed that the mycelium initiation from the inoculated agar block took few hours in case of agar block method whereas, in case of typha bit

Table 1: Differences between the two inoculation techniques at mass multiplication phase

Method	Mycelial initiation (dpi)	Time taken to cover walls/ plate(dpi)	Time taken to form sclerotia (dpi)	Total duration for mass multiplication(dpi)
Agar Block	1	4	8	10
Typha Bit	2	6	15	20

Table 2: Differences between the two inoculation techniques at inoculation phase

Method	Incubation Period (dpi)	Lesion Height (cm)	Plant Height (cm)	RLH %	Grade	Disease Reaction
Agar Block	4th	39.72	56.42	70.40	9	HS
Typha Bit	7th	37.32	54.70	68.22	9	HS

* Lesion height and plant height recorded in this study are average of five replications

inoculation method, first visible growth was seen after two days. In case of agar block method, the entire 90 mm petriplate was covered with mycelia in 3-4 days whereas; the walls of the conical flask were covered with whitish mycelium after 5 dpi. The RS4 culture formed scattered whitish sclerotia within six days after subculturing, which turned brown at 7th to 8th day onwards in case of culture preparation by agar block method. The same culture took ten to twelve days to form whitish sclerotia on the typha bits which turned brown fifteen days after inoculation (Table 1). This may be possible because the agar block was in direct contact with substrate in agar block method which supports easy colonization, growth and development compared to typha bit method. Moreover, the moist cotton and aluminium foil used in the agar block method helps to avoid the variation in disease development and reduces environmental effects.

The entire duration for mass multiplication of RS4 took ten days in case of agar block method and nearly double in case of colonized typha bit method including two days for consecutive autoclaving (Table 1).The inoculation procedure was comparatively cumbersome in agar block method compared to typha method with the former taking more time to inoculate each plant with rice ShB pathogen. The agar block method took four days to produce symptom on susceptible host BPT-5204 after inoculation, whereas typha method took seven days to initiate first visible symptom (Table 2). The density of the primary inoculum is a major factor governing ShB disease outbreak in rice. The disease incidence as well as severity was reported to be quite high in their study, when the inoculation was carried out with mycelia and sclerotia (Khoshkdaman *et al*., 2020).

The lesions were oval to oblong shaped in case of BPT-5204 plants inoculated with RS4 using agar block method (Fig. 4A) whereas, the lesions were irregular in case of plants inoculated with typha bit method (Fig. 4B). The symptoms produced as a result of colonized typha bit method were uniformly present over the sheath portion only whereas, the symptoms due to RS4 inoculation by agar block method were present on sheath as well as leaf portions in a scattered manner at 7 dpi (Fig. 4A, 4B).The average lesion height and RLH % on 21 dpi was higher in case of agar method compared to typha method proving that the disease severity was more in the former (Table 2). The RLH percentage obtained in both these methods was above 66 per cent which represents highly susceptible (HS) disease reaction as per SES (IRRI, 2002)

The colonized typha bit method has been previously used in many studies to identify the resistant and moderately resistant genotypes against rice sheath blight such as the study conducted by Dubey *et al*. (2014), wherein four rice genotypes, *viz*., BPL 7-12, BML21-1, BML27-1 and Kajarawaha were found to be tolerant to ShB out of hundred genotypes screened under field conditions, Yadav *et al*. (2015), wherein Tetep and ARC 10531 were found to be moderately resistant among the forty germplasms screened, Turaidar *et al*. (2017), wherein Bangara Sana was found to be moderately resistant amongst thirty landraces tested against ShB and Samal *et al*. (2022), wherein, out of seventy genotypes screened only CR 1014 and Tetep showed moderately resistant reaction against *Rhizoctonia solani* Kuhn. Similarly, the agar block method had also been used by many researchers such as Jia *et al*. (2011), wherein fifty two USDA rice accessions showed significantly more resistance against rice ShB pathogen out of the one thousand seven hundred and ninety four rice accessions screened, Lavale *et al*. (2018), wherein the landrace Nizam Shait was found to be the only resistant landrace among one hundred thirty four landraces screened, Goswami *et al*. (2019), wherein two

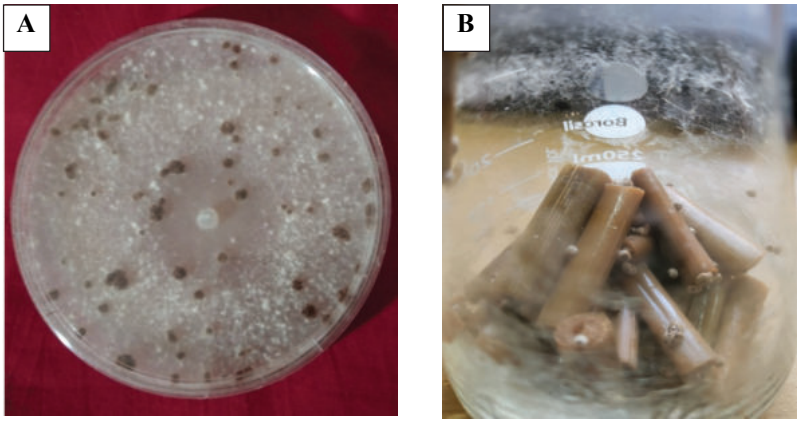

Fig. 1: Mass multiplication of RS4 isolate of *Rhizoctonia solani*
A. on PDA where brown colour depicts mature sclerotia
B. on typha bits where white mycelium is observed on the walls of conical flask and sclerotia on the typha bits

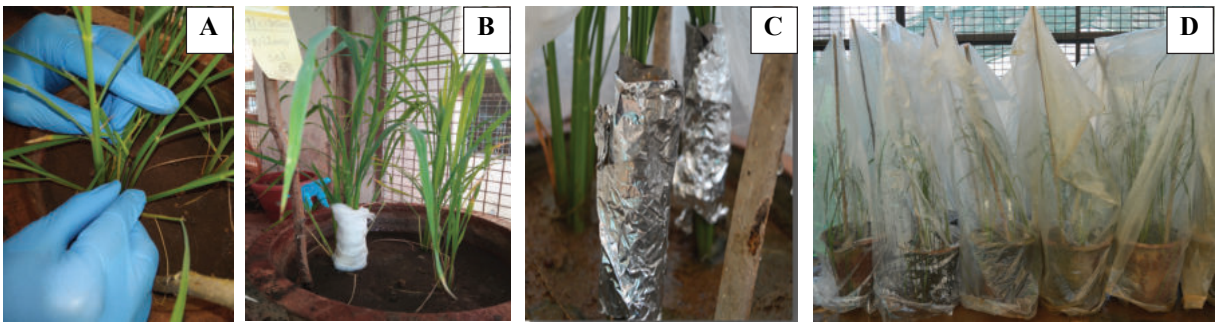

Fig. 2: Steps involved in agar block inoculation method
A) Opening the sheath and placing agar block with sclerotia B) Covering inoculated portion with wet cotton
C) Wrapping aluminium foil around wet cotton tightly D) Covering the inoculated plants with polythene cover

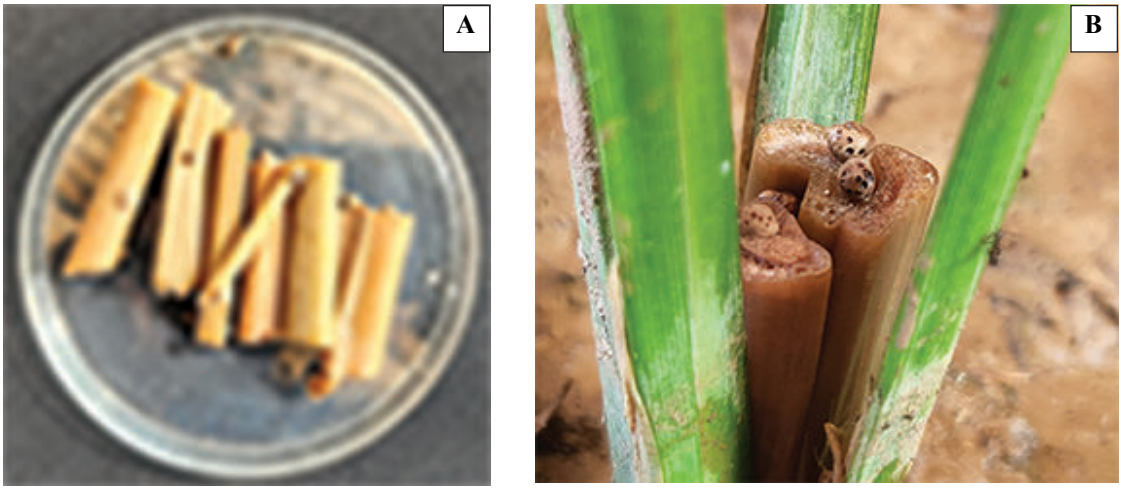

Fig. 3: Steps involved in colonized typha bit inoculation method
A) Typha bits covered with mycelia and sclerotia of RS4
B) Colonized typha bits placed at the centre of rice tillers

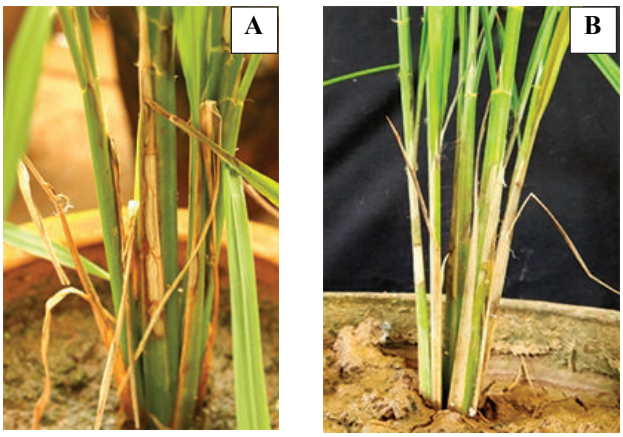

Fig. 4: Symptoms observed on BPT-5204 after inoculating with RS4 isolate of *Rhizoctonia solani* on 7th dpi by
A) Agar block method where sheath as well as leaves were affected and
B) colonized typha bit inoculation method where symptoms were observed on sheath portion

hundred and sixty-one germplasm lines were screened using mycelia bits or immature sclerotia and fifty seven were reported to be resistant against rice ShB and Gupt *et al*. (2020), wherein forty two advanced lines of rice were evaluated against rice ShB artificially and three genotypes, namely, GSR 310, Hardinath-3 and Sabitri were moderately resistant. A detailed comparison of the inoculation methods serves as an important resource for future studies aimed at screening for ShB resistance. Park *et al*. (2008) reported that the ShB inoculation carried out using mycelial ball led to a higher infection rate compared to the mycelial disc and suspension. A report by Kumar *et al.* (2019) stated that the ShB disease incidence was maximum in sclerotial inoculation method compared to soil inoculation using homogenized mycelia suspension. Lore *et al*. (2021) used the three most common sheath blight inoculum types, *viz*., mycelial bit, single sclerotium and mycelial ball and observed that the mycelial balls produced significantly higher level of disease in the rice plants tested. The type of inoculum used in ShB evaluation influences the disease development and in turn compromises the decision to categorize the level of resistance in the evaluated material (Lore *et al*., 2021). However, Singh *et al*. (2001) stated that the most critical factor determining the uniform development of ShB infection was the amount of inoculum and not the type of inoculum used. Though it is difficult to underscore the relative importance of these two factors, it is evident that both the factors affect the rate of ShB infection. Previous studies used lesion length parameters and visual rating scales to check the ShB disease severity; however, the inoculation methods have not been compared side by side to assess their accuracy (Park *et al*., 2008). So, this is the first study of its kind wherein the two most commonly used techniques of rice ShB inoculation have been studied in detail at mass multiplication and inoculation level.

CONCLUSION

Identification of resistant varieties of rice is the most economical way of managing the rice sheath blight disease. Hence, effective inoculation methods are must for screening the available germplasm against rice ShB, measuring the disease severity and for identifying the new races of the pathogen.The rice sheath inoculation with agar block along with sclerotia and inoculation of base of the tiller with typha bits have been proven to be the two most efficient, consistent and reproducible methods of rice sheath blight disease inoculation under field and artificial conditions. Both of these have their own advantages and disadvantages. Mass multiplication for agar block method is easier compared to colonized typha bit method and it can be used for small scale inoculation in artificial conditions and studies based on omics. Colonized typha bit method is more suitable for mass screening of germplasms in field and artificial conditions as it requires less time to inoculate the plant. The typha plants are not easy to find everywhere. Hence, agar block method can be preferred at such locations.

REFERENCES

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A.P.K. and John,V.T. 1978. Evaluation for sheath blight resistance in rice. *IRRN*, International Rice Research Institute, Manila, Philippines.

Che, K., Zhan, Q., Xing, Q., Wang, Z., Jin, D., He, D. and Wang, B. 2003. Tagging and mapping of rice sheath blight resistant gene. *Theor. Appl.Genet.*, **106**: 293-297.

Dubey, A.K., Pandian, R.T.P., Rajashekara, H., Singh, V.K., Kumar, G., Sharma, P., Kumar, A., Gopala Krishnan, S., Singh, A.K., Rathour, R. and Singh, U.D. 2014. Phenotyping of improved rice lines and landraces for blast and sheath blight resistance. *Indian J. Genet.*, **74**: 499-501.

FAO. 2021. Area and production of Rice during 2019-2020 https://www.fao.org/faostat/en/#data/QCL/visualize (Last accessed on 15 September, 2022).

Goswami, S.K., Singh,V., Kashyap, P.L. and Singh, P.K. 2019. Morphological characterization and screening for sheath blight resistance using Indian isolates of *Rhizoctonia solani* AG1IA. *Indian Phytopathol.*, **72**: 107-124.

Gupt, S.K., Pant, K.R., Basnet, R., Yadhav, M., Pandey, B.P. and Bastola, B.R. 2021. Assessment of rice genotypes to susceptibility of sheath blight disease caused by *Rhizoctonia solani* AG1-IA. *FAA*, **6**: 57-66.

IRRI. 2002. *Standard Evaluation System for Rice*. International Rice Research Institute, Manila, Philippines. pp 56.

Jia, L., Yan, W., Agrama, H.A., Yeater, K., Li, X., Hu, B., Moldenhauer, K., McClung, A. and Wu, D. 2011. Searching for germplasm resistant to sheath blight from the USDA rice core collection. *Crop Sci.*, **51**: 1507 -1517.

Jia,,Y., Correa-Victoria, F., McClung, A., Zhu, L., Liu, G., Wamishe, Y., Xie, J., Marchetti, M.A., Pinson, S.R. M., Rutger, J.N. and Correll, J.C. 2007. Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. *Plant Dis.*, **91**: 485-489.

Khoshkdaman, M., Mousanejad, S., Elahinia, S.A., Ebadi, A. and Padasht-Dehkaei, F. 2020. Impact of soil-borne inoculum on sheath blight disease development in rice. *J. Crop Prot.*, **9**: 625-635.

Kumar, S., Akhtar, M.N. and Kumar, T. 2019. Standardization of inoculation techniques for sheath blight of rice caused by *Rhizoctonia solani* (Kuhn). *Bangladesh J. Bot.*, **48**: 1107-1113.

Lavale, S.A., Prashanthi, S.K. and Fathy, K. 2018. Mapping association of molecular markers and sheath blight (*Rhizoctonia solani*) disease resistance and identification of novel resistance sources and loci in rice. *Euphytica*, **214**: 78.

Li, D., Zhang, F., Pinson, S.R., Edwards, J.D., Jackson, A.K., Xia, X. and Eizenga, G.C. 2022. Assessment of rice sheath blight resistance including associations with plant architecture, as revealed by genome-wide association studies. *Rice*, **15**: 31.

Lore, J.S., Jain, J., Hunjan, M.S., Kamboj, I., Zaidi, N.W. and Singh, U.S. 2021. Efficiency of axenically grown *Rhizoctonia solani* inoculum types in evaluating sheath blight development on rice genotypes. *J. Phytopathol.*, **169**: 508– 513.

Miyake, I. 1910. Studienuber die Pilze der Reispflanze in Japan. *J. Coll. Agric. Imp. Univ. Tokyo*, **2**: 237-276.

Park, D.S., Sayler, R.J., Hong, Y.G., Nam, M.H. and Yang,Y.A. 2008. A method for inoculation and evaluation of rice sheath blight disease. *Plant Dis.*, **92**: 25–29.

Prasad, B. and Eizenga, G.C. 2008. Rice sheath blight disease resistance identified in *Oryza* spp. accessions. *Plant Dis*, **92**: 1503-1509.

Samal, P., Molla, K.A., Bal, A., Ray, S., Swain, H., Khandual, A., Sahoo, P., Behera, M., Jaiswal, S., Iquebal, A. and Chakraborti, M. 2022. Comparative transcriptome profiling reveals the basis of differential sheath blight disease response in tolerant and susceptible rice genotypes. *Protoplasma*, **259**: 61-73.

Singh, A., Rohila, R., Singh, U.S., Savary, S., Willocquet, L. and Duveiller, E. 2001. An improved inoculation technique for sheath blight of rice caused by *Rhizoctonia solani*. *Can. J. Plant Pathol.*, **24**: 65–68.

Sivalingam, P.N., Vishwakarma, S.N. and Singh, U.S. 2006. Role of seed-borne inoculum of *Rhizoctonia solani* in sheath blight of rice. *Indian Phytopathol.*, **59**: 445-452.

Skamnioti, P. and Gurr, S.J. 2009. Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol*, **27**: 141-150.

Statista. 2021. **Rice** - Statistics & Facts 2020-2021 https://www.statista.com/topics/1443/rice (Last accessed on 28 August, 2022).

Suryawanshi, P.P., Krishnaraj, P.U. and Prashanthi,S.K. 2019. Morphological and molecular characterization of *Rhizoctonia solani* causing sheath blight in rice. *Int. J. Curr.Microbiol. Appl.Sci.*, **8**: 1714-1721

Turaidar, V., Krupa, K.N., Reddy, M., Deepak, C.A., Harini, K.K.M. and Subhash, B.S. 2017. Phenotyping of rice landraces for sheath blight resistance. *J Pharmacog. Phytochem.*, **6**: 2209-2212.

Wasano, K. and Hirota, Y. 1986. Varietal differences in the resistance to sheath blight disease caused by *Rhizoctonia solani* Kuhn, by the syringe inoculation methods. *Bull. Faculty Agric. Saga Univ.*, **60**: 49-59.

Wu, W., Huang, J., Cui, K., Nie, L., Wang, Q., Yang, F., Shah, F., Yao, F. and Peng, S. 2012. Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. *Field Crops Res.*, **128**: 101–108.

Yadav, S., Kumar, R.R., Anuradha, G., Reddy, V.L.N. and Sudhakar, R. 2015. Screening of rice germplasms for sheath blight resistance and assessment of parental polymorphism using SSR markers. *Ecol. Environ. Conserv.*, **21**: 295-301.

Yeh, W.H. and Bonman, J.M. 1986. Assessment of partial resistance to *Pyricularia oryzae* in six rice cultivars. *Plant Pathol.*, **35**: 319-323.

Zou, J. H., Pan, X.B., Chen, Z.X., Xu, J.Y., Lu, J.F., Zhai, W.X. and Zhu, L.H. 2000. Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.).*Theor. Appl. Genet.*, **101**: 569-573.