CREATING BROAD-SPECTRUM DISEASE RESISTANCE AGAINST RICE BLAST BY THE INTRODUCTION OF A NON-HOST RESISTANCE GENE FROM ARABIDOPSIS INTO RICE

Rishi Kesh; Binod Bihari Sahu*

Department of Life Science, NIT ROURKELA, ODISHA-769008

Email: sahub@nitrkl.ac.in

Rice is grown extensively in many parts of the world at different times of the year under different climatic conditions. Rice is the most essential cereal crop that forms a major part of the human diet and is hence popularly called "Global grain". With the increasing trend of the rising human population, it’s high time to have crops with high yield potential and stability. Making the rice-based production system sustainable is crucial for the future food security of mankind. Rice blast is one of the fungal diseases that hamper the rice crop production by farmers, which could feed 6 million people a year. Rice blast is generally observed in the area where rice is cultivated. However, farmers’ use of chemical pesticides to curtail the infection helps the pathogens to evolve into new races with enhanced resistance and that affects the environment greatly.

Non-host resistance genes from the evolved plant in nature provide broad spectrum resistance as they can resist all forms of pathovars (Heath, 2000). Arabidopsis is immune to *M. oryzae* and is considered a nonhost plant possibly due to the presence of functional nonhost resistance genes in it (Park et al., 2009, Nakao et al., 2011). By following forward genetics, we identified that mutation as SNP (Ala to Val) in an NHR gene, *MOSA* in Arabidopsis leads to a breach in nonhost resistance against rice blast. Further, from a screening of 14 *pss* mutants of Arabidopsis, we identified that SNP mutation in specific genes also breached NHR against rice blast. We are aiming to create transgenic rice by introducing one of the screened genes from the mutant Arabidopsis via the Agrobacterium-mediated gene transfer method in the embryogenic elite cvr rice calli. Further, evaluating the promoter activity of the identified NHR gene upon infection in this transgenic line would delineate the plausible mode of genetic control in the activation of nonhost resistance against non-adapted pathogens that can be used in generating novel germplasm.

Keywords: non-host resistance, rice blast, agrobacterium, embryogenic, transgenic

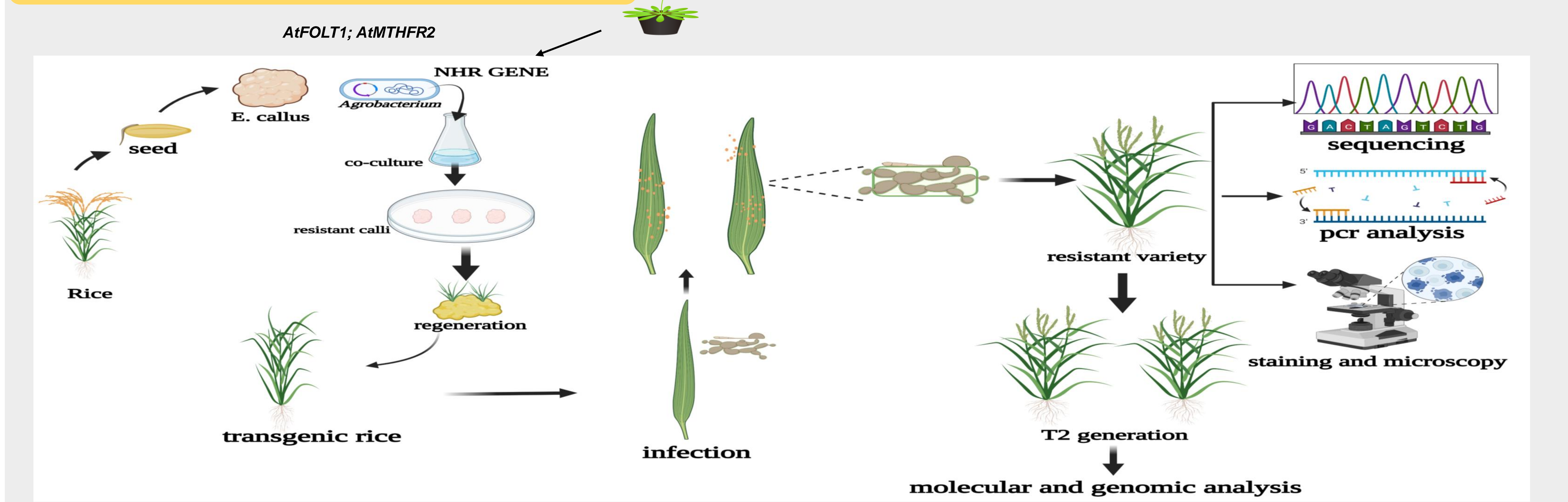
Enhancing broad-spectrum disease resistance against rice blast by engineering orthologous nonhost resistance genes of Arabidopsis in rice

Rishi Kesh and Binod Bihari Sahu
Email: 521LS6008@nitrkl.ac.in
Laboratory Of Molecular Genetics And Plant Immunity, Department Of Life Science
National Institute Of Technology, Rourkela, Odisha, 769008

Abstract

Rice is grown extensively in many parts of the world at different times of the year under different climatic conditions. Rice is the most essential cereal crop that forms a major part of the human diet and is hence popularly called "Global grain". With the increasing trend of the rising human population, it's high time to have crops with high yield potential and stability. Making the rice-based production system sustainable is crucial for the future food security of mankind. Rice blast is one of the fungal diseases that hamper the rice crop production by farmers, which could feed 6 million people a year. Rice blast is generally observed in the area where rice is cultivated. However, farmers' use of chemical pesticides to curtail the infection helps the pathogens evolve into new races with enhanced resistance, affecting the environment greatly. Non-host resistance genes from the evolved plant in nature provide broad spectrum resistance as they can resist all forms of pathovars (Heath, 2000). Arabidopsis is immune to *M. oryzae* and is considered a nonhost plant possibly due to the presence of functional nonhost resistance genes in it (Park et al., 2009, Nakao et al., 2011). By following forward genetics, we identified that mutation as SNP (Ala to Val) in an NHR gene, *MOSA* in Arabidopsis leads to a breach in nonhost resistance against rice blast. Further, from a screening of 14 mutants of Arabidopsis, we identified that SNP mutation in specific genes also breached NHR against rice blast. We aim to create transgenic rice by introducing one of the screened genes from the mutant Arabidopsis via the Agrobacterium-mediated gene transfer method in the embryogenic elite CVR rice calli. Further, evaluating the promoter activity of the identified NHR gene upon infection in this transgenic line would delineate the plausible mode of genetic control in the activation of nonhost resistance against non-adapted pathogens that can be used in generating novel germplasm.

Rationale & Objectives



Materials and methods

A. Seeds and plant growth Conditions.
Seeds of elite rice cv. Swarna was obtained from NRRI, Cuttack, Odisha. Arabidopsis seeds (Col-0, T-DNA insertion lines) were obtained from NASC and mosA seeds were harvested from EMS generation. pss30 seeds were obtained from Iowa State University, USA. Arabidopsis seedlings were grown in the walk-in plant growth chamber under standard growth conditions.

B. Optimization for generation of embryonic calli of elite rice cv.
Swarna. Mature and healthy seeds were dehusked and standard surface sterilized and inoculated on MS media supplemented with MS vitamin, 2mg/l 2,4-D, 0.5g/l casein hydrolysate, 0.6g/l L-proline, 30g/l maltose solidified with 0.4% clerigel and pH 5.8 and was incubated in dark at 28°C for 2-3 weeks for callus initiation.

C. Optimizations of regeneration media for shoot and root generation from the obtained calli.
Embryonic Calli were transferred to MS media supplemented with 2mg/L kinetin, 0.2mg/L NAA, maltose 30g/L, MS vitamin, with 0.8% agar and incubated under dark at 28°C for seven days. Greenish calli thus seen by 4 days were transferred onto regeneration media and kept under a light photoperiod. As the shoots elongated up to 6-7 cm in length, they were transferred to rooting media containing half the strength of MS basal salt.

D. Cloning of NHR GENE into pCAMBIA1301.

E. Transformation of cloned NHR genes into Agrobacterium *LBA4404 and EHA105*

F. Screening the phenotypes by microscopy of *M. oryzae* infected Arabidopsis leaves.
Leaves of Arabidopsis mutant for NHR gene were infected with *M. oryzae* and further examined for cell death by Trypan blue staining in pss30, ROS accumulation by DAB staining, and Quantitative analysis of penetration resistance to *M. oryzae* in Arabidopsis having NHR GENE.

G. Cloning of the promoters of NHR GENE from Arabidopsis and rice.
Various sizes of the promoter region of the respective genes from Arabidopsis and rice were cloned directionally in pCAMBIA1301 which were transformed into Agrobacterium.

Results

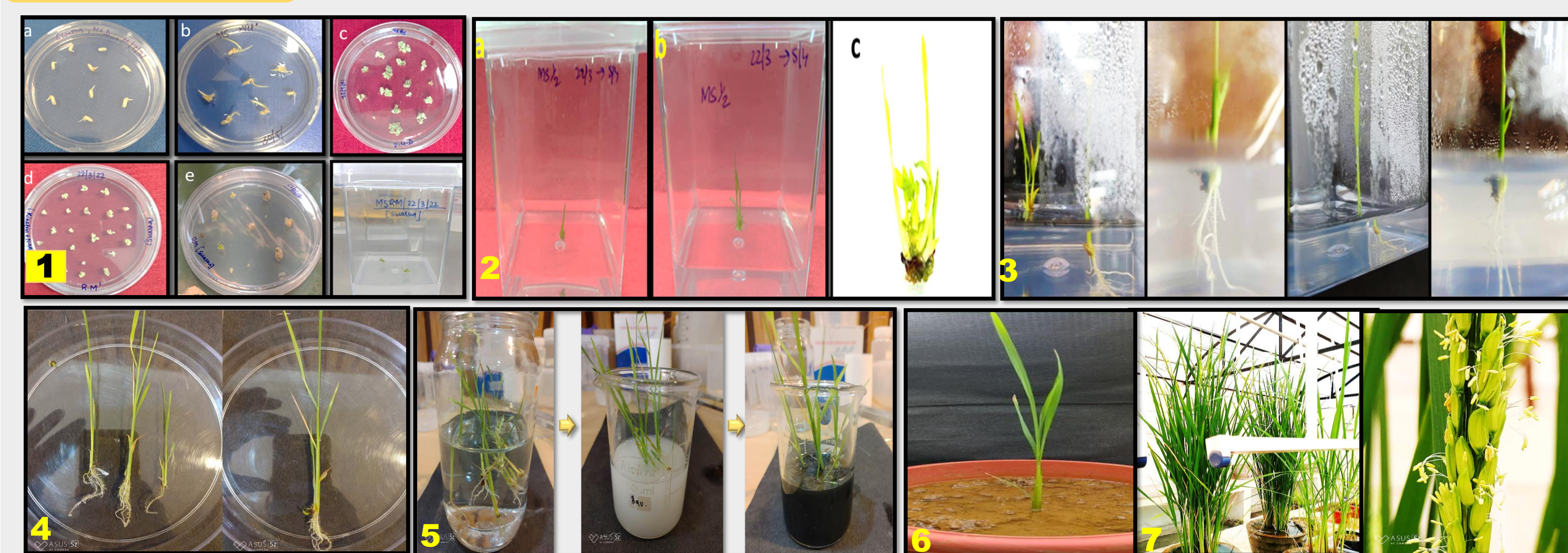


Fig.1. Generation of embryonic calli; sterilized rice seed inoculated on callus media after (a) 3 days (b) 7 days (c) 3 weeks (d) transfer of calli to regeneration media (e) greenish spot appearance on calli after continuous light for 4 days (f) shoots initiated from the greenish calli Fig.2. Induction of shoot from emerged shoots to rooting media (c) multiple shoots regenerated from calli Fig.3. Induction of rice root tissue (after 1 week) from regenerated shoots placed in MS1/2 media kept at 28°C Fig 4; Recovering of rooted plantlets from the rooting media Fig.5. Preparation of plantlets before transferring to the soil for hardening Fig.6: Fully acclimatized rice plantlets after 2 weeks in the growth chamber under 16/8 hrs photoperiod before transferring it to the greenhouse. Fig.7. Maturing rice plant

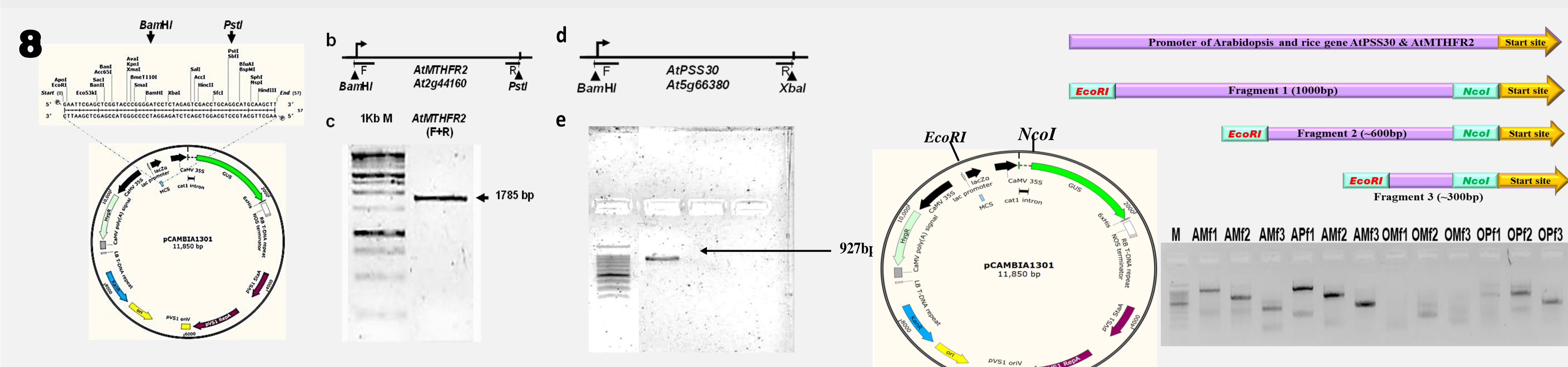


Fig.8.Cloning of NHR genes from Arabidopsis along with the homologous gene's promoters from rice in pCAMBIA1301

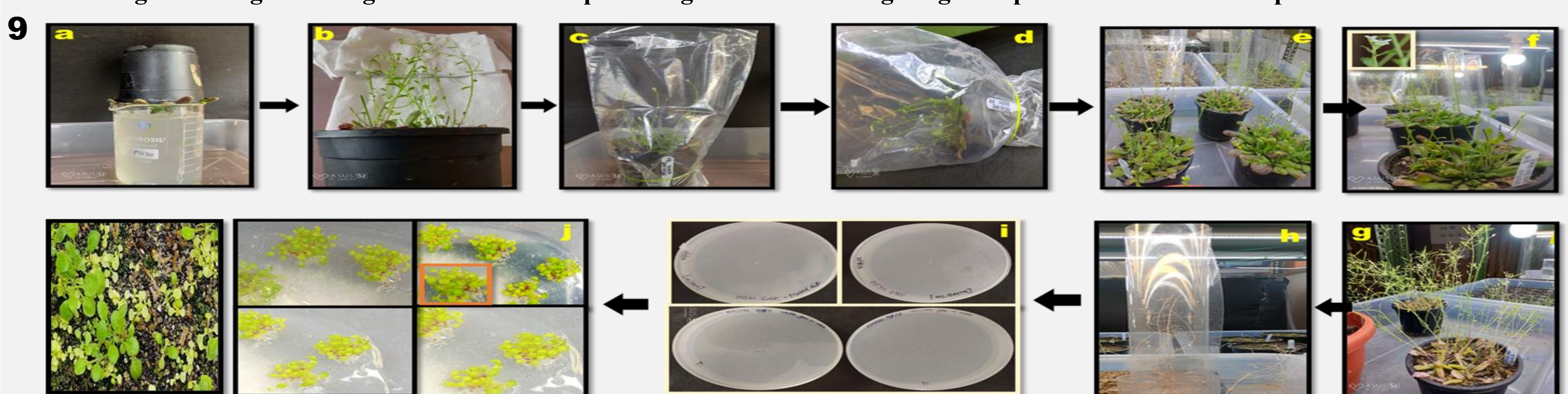


Fig. 9. Floral dip of Arabidopsis mutant pss30 ems and pss30 salk line with agrobacterium containing *AtFOLT1 gene and MOSA with AtMTHFR2 gene*

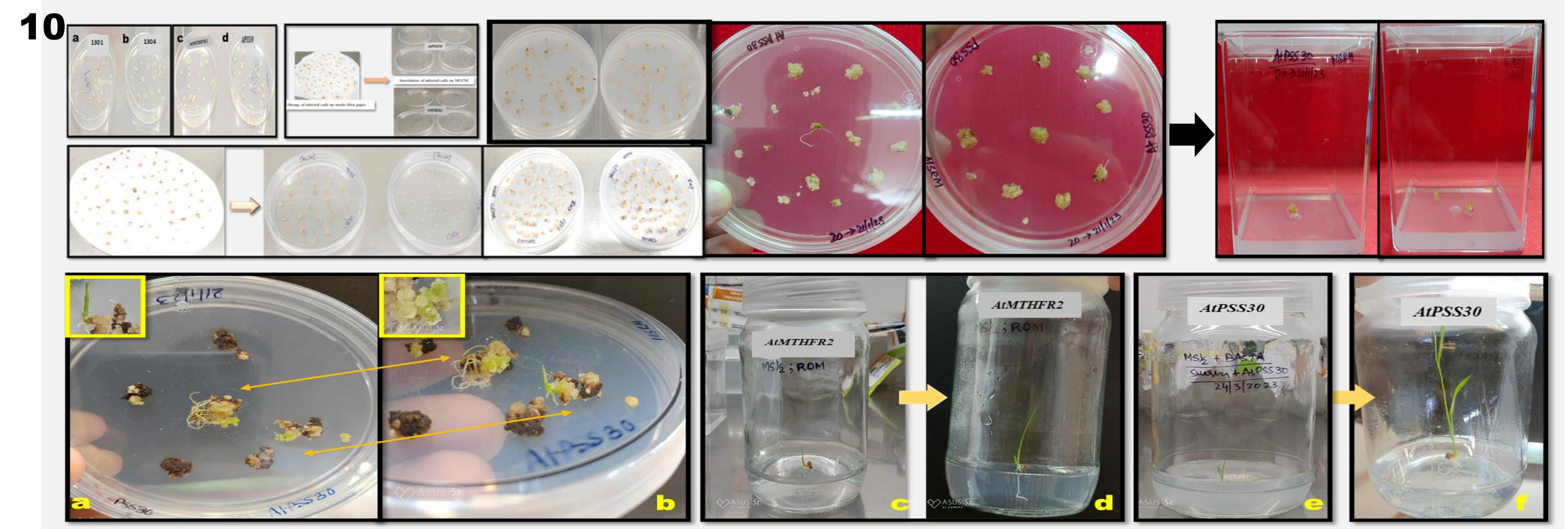


Fig. 10. Agrobacterium-mediated transformation of embryonic calli to transfer NHR gene *AtFOLT1* and *AtMTHFR2*

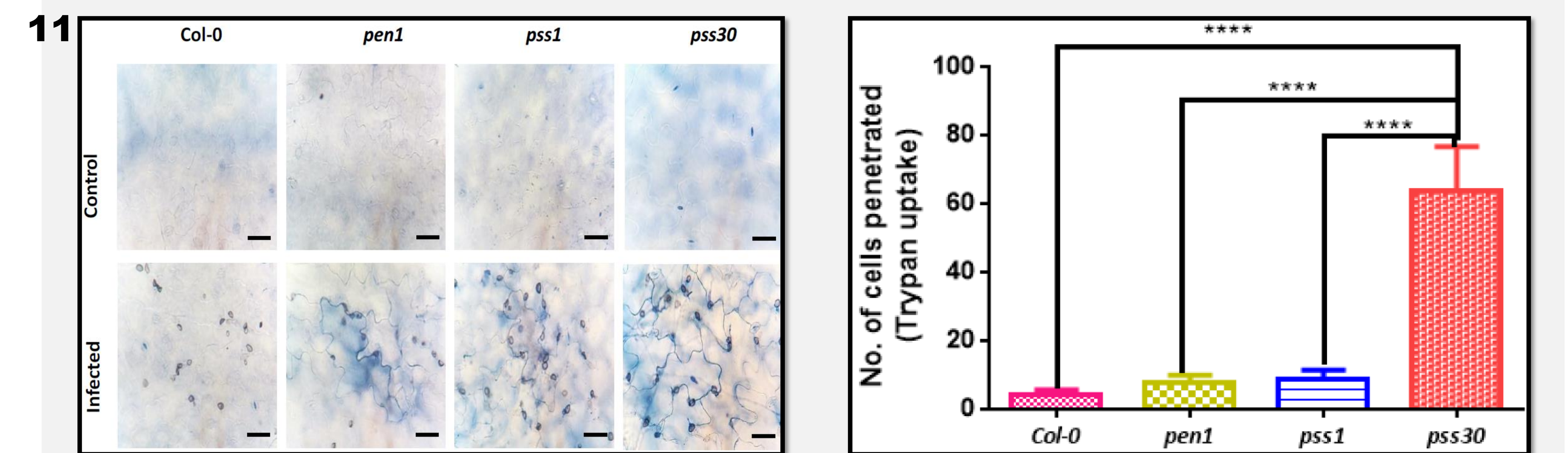


Fig. 11. Cell death observation by Trypan blue staining in *pss30*. Arabidopsis leaves infected with *M. oryzae* were harvested after 3 dpi and stained with trypan blue to observe cell death in penetrated cells under a bright field microscope. Leaves inoculated with 0.01% Tween 20 served as control. *pen1* and *pss1* (EMS generated mutant in *pen1* background) were kept as control. Bars = 50µm. Fig.10. Quantitative analysis of penetration resistance to *M. oryzae* in Arabidopsis *pss30*. Mean penetration in *A. thaliana* wild type and mutants after 3 days post inoculation (dpi), as observed by Trypan blue uptake by penetrated cells. Pen1 is used as a background control and *pss1* is an EMS mutant. Data represent mean ± standard error of at least two independent experiments. Asterisks indicate a significant difference in paired comparison ($P < 0.05$); Tukey's multiple comparison test.

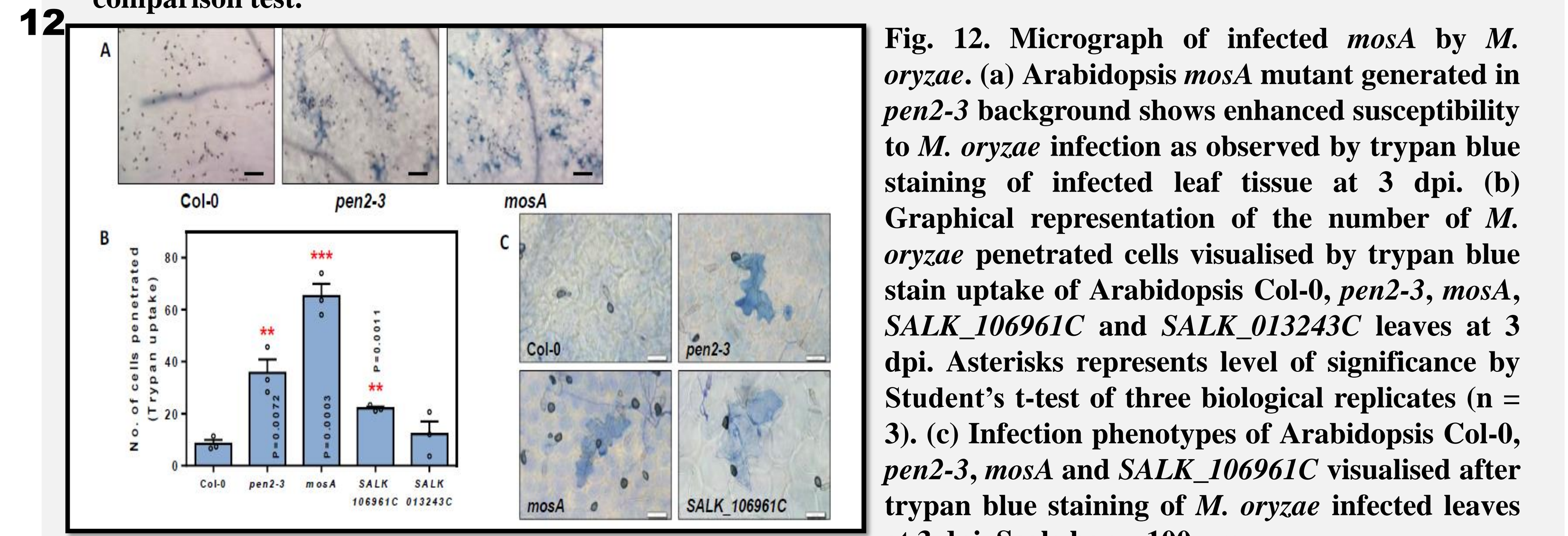


Fig. 12. Micrograph of infected *mosA* by *M. oryzae*. (a) Arabidopsis *mosA* mutant generated in *pen2-3* background shows enhanced susceptibility to *M. oryzae* infection as observed by trypan blue staining of infected leaf tissue at 3 dpi. (b) Graphical representation of the number of *M. oryzae* penetrated cells visualised by trypan blue stain uptake of Arabidopsis Col-0, *pen2-3*, *mosA*, *SALK_106961C* and *SALK_013243C* leaves at 3 dpi. Asterisks represents level of significance by Student's t-test of three biological replicates (n = 3). (c) Infection phenotypes of Arabidopsis Col-0, *pen2-3*, *mosA* and *SALK_106961C* visualised after trypan blue staining of *M. oryzae* infected leaves at 3 dpi. Scale bar = 100 µm.

Conclusion

- It was found that the synthetic auxin, 2,4-D at a concentration of 2 mg/L was suitable for callus initiation in rice (Swarna cv.).
- For regeneration, 2mg/L kinetin along with 0.2 mg/L NAA was appropriate.
- During the regeneration, gelling agent agar 0.8 % showed improved frequency when compared to 0.4 % clerigel.
- Incubating calli in regeneration media under dark for 7 days and then transferring to continuous light for 4-5 days played a significant role in turning the calli greenish.
- Half strength of basal MS medium is enough for rooting the emerged shoots from calli.
- Cloning of the full-length genes of *AtMTHFR2* and *AtPSS30* was performed successfully
- Cloning of series of promoters' fragments of the two nonhost resistance genes in binary vector and their transformation in Agrobacterium were successful

Future Aspects

- The generated germplasm of rice would provide sustainable nonhost resistance against rice blast in filed condition thereby increasing the crop yield
- We understand the biochemical and molecular mechanism of disease resistance governed by the NHR genes (PSS30 and AtMTHFR2) that can be implemented.
- The constructs of binary vectors encompassing the two NHR genes and the promoter series are to be transformed in Arabidopsis and rice and the efficacy of disease resistance incurred due to the transgene will be tested and characterized further.

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