Identifying the components in Spl11-mediated defense pathway and determining the relationship between Spl11 and other defense signaling genes in rice

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Abstract
Ubiquitination has recently been shown to be involved in programmed cell death (PCD) in plants. Spl11 encodes an E3 ubiquitin ligase with U-Box and ARM repeat domains which negatively regulates PCD and disease resistance in rice. To identify new components in Spl11-mediated cell death pathway, a suppressor screen was performed using the suppressor line and EMS as a mutagen. The spontaneous lesion formation observed in GR5717 was found to be completely suppressed in one suppressor, whereas, two other suppressors showed partial suppression. Broad-spectrum resistance in GR5717 was found to be abolished in all the suppressors. To map the genes involved in this suppression phenomenon, three F2 mapping populations were generated using the suppressor lines and spl11 mutant line TP309spl11. Two F2 populations show 3:1 ratio of segregation for suppression phenotype to lesion mimic phenotype whereas one shows 13:3 ratio, indicating either single-gene Mendelian inheritance or inheritance of one dominant and one recessive genes. Simultaneously F2 populations are also being generated to test for allelism among different suppressors. Further, genetic analysis to study the relationship between Spl11 and other defense signaling genes such as NPR1, SGT1, RAR1 and Rac1 has been undertaken. Crosses between spl11 knockdown or Spl11 overexpression lines and other defense mutants are being generated. Overall, ubiquitination mediated defense mechanisms will be elucidated through these studies in rice which is the world’s most important food crop.

Introduction
Ubiquitination is emerging as an important cellular event which is involved in diverse biological processes in plants, such as development and responses to abiotic and biotic stresses (Vierstra, 2003; Zeng et al., 2006). Identification of the Spl1 gene’s function in negative regulation of programmed cell death (PCD) in plants provided a direct link between PCD and/or disease resistance and ubiquitination. Cloning of Spl11 gene’s function in negative regulation of programmed cell death (PCD) in plants will help in understanding the role of ubiquitination in disease resistance.

Results

1. Identification of suppressor lines of spontaneous lesions caused by spl11

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Suppressor screen was performed on GR5717 (containing spl11 mutation) using EMS and one full suppressor line (963) and two partial suppressors (1902 and 6455) were generated which showed either no or highly reduced spontaneous lesion formation.

Further RFLP analysis followed by Southern blotting excluded the possibility of spl11 revertant mutations.

2. Response of suppressor lines to Xanthomonas oryzae pv. oryzae (Race 6)

All the suppressor lines show loss of resistance to Xanthomonas oryzae pv. oryzae and Magnaporthe grisea (data not shown) which was typical to the lines carrying spl11 mutation, suggesting probable role of those suppressor genes in cell death pathway related to pathogen resistance.

3. Genetic analysis of suppressor gene/s

In order to determine the number of genes involved in the phenomenon of suppression of spontaneous lesion formation and to initiate the mapping of the gene/s, we made crosses between TP309spl11 and suppressor lines 963, 1902 and 6455. All the F1 plants were suppressed suggesting dominant nature of the mutations. 3:1 (suppressed: non-suppressed lesion mimics) ratio was observed in F2 population derived from crosses made between TP309spl11 and 1902 and 6455, indicating inheritance of single dominant mutation in either of those suppressor lines. Ratio of 13:3 (suppressed: non-suppressed lesion mimics) was observed in F2 progeny derived from cross between 963 and TP309spl11, suggesting inheritance of one dominant and one recessive mutation.

Conclusions
• We identified three suppressor lines by performing suppressor screening on GR5717 (spl11) using Ethyl Methane Sulfonate as Mutagen
• All the suppressor lines show abolishment of resistance to Xanthomonas oryzae pv. oryzae and Magnaporthe grisea
• Suppressor line 1902 and 6455 show involvement of one dominant mutation in the suppression whereas line 963 shows involvement of one dominant and one recessive mutation manifesting in the suppression.

Future Directions
• The F2 populations generated for the genetic analysis are currently being used for mapping the gene/s involved in the suppression phenomenon.
• We are currently testing F2 populations generated from crosses among different suppressor lines to test for allelism between the gene/s involved in the suppression.
• In order to determine the role of Spl11 in different defense related pathways in rice, we are currently creating double mutants by using spl11 and mutant lines of various genes shown to be involved in defense pathways in rice such as OsSGT1, OsRAR1, OsRac1, OsMapk5, OsWmk1-1, OsEin2. Also, we will be using lines over-expressing OsNRR, OsNHT, OsRac1 to make crosses with spl11 line. We have also generated a line over-expressing Spl11 (TP309OxSpl11) by Agrobacterium tumefaciens mediated transformation of rice. We will also be using this line to make the crosses with all the above mentioned lines for our studies.

Acknowledgements
The authors thank Qi Sun and Maria Bellizzi for their technical assistance. This work was supported by grants from the National Research Initiative of the USDA Cooperative State Research Education and Extension Service #2007-01967 (GLW); USAID/IRRI Linkage Program (GLW and HL)

References: