Optimizing Cellulase RS and Macerozyme R-10 Ratios to Maximize Viable Protoplast Yield from Oryza sativa: Improving the Process of Creating Genetically Modified Plants

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The purpose of this project was to was to find the optimal mixture of two enzymes, Cellulase RS and Macerozyme R-10, in order to maximize protoplast yield, viability, and size. Plant protoplasts are plant cells without cell walls. These cell walls are digested by the two enzymes tested in this experiment, cellulase and macerozyme. Protoplasts are very useful in the field of agricultural biotechnology, as they are the basis for the genetic modification of all plants. Therefore, by increasing the yield of viable and healthy protoplasts, the process of creating transgenic plants will be made more efficient. Currently, one particular enzyme solution, which served as the control group in this study, is made up of a 2% mixture of 75% cellulase and 25% macerozyme. This is the solution typically used to isolate protoplasts from Oryza sativa, or rice; however, it has not been tested to see whether it is the most effective mixture. Therefore, five other enzyme solutions were tested, which had varied ratios of cellulase and macerozyme and different amounts of the overall mixture of only cellulase and macerozyme. Protoplasts from rice leaves were isolated using the control enzyme solution and the five others. Data was gathered on protoplast yield, viability, and size to create equations which showed the optimal ratio of the two enzymes. The enzyme solution made up of a 4% mixture of 50% cellulase and 50% macerozyme released the highest amount of protoplasts and the highest percentage of viable protoplasts. This enzyme solution had a protoplast yield that was more than three times the yield of the control group. With this improved enzyme solution, protoplast isolation is made much more efficient.