Identifying the Key Mechanisms Behind Successful Seedling Emergence in Rice

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INTRODUCTION

Rice is one of the world's most important crops accounting for a total global production of 480 million tons a year. Billions of people rely on this staple crop to sustain life. However, as the world population continues to grow towards the projection of ten billion people by 2050, it is becoming difficult to achieve global food security. In addition, climate change is currently destroying arable land and hindering global rice production.
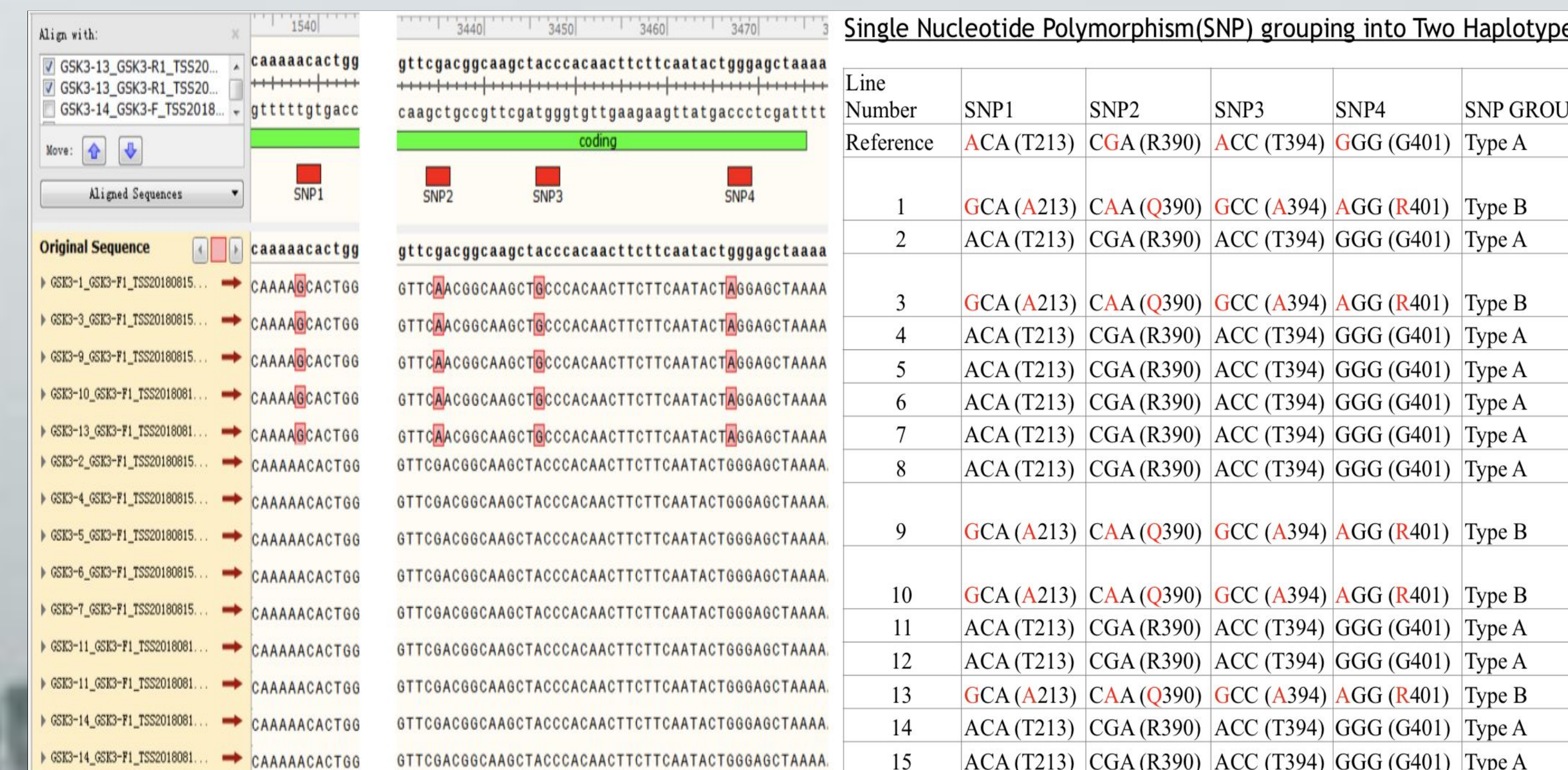
The traditional method to grow rice requires a gargantuan amount of time, effort, and, most importantly, water. Due to this, many alternative methods have been explored to use resources more efficiently and ultimately increase crop yield. The direct seed sowing method has proven to be quite successful in saving time and water. However, there are many disadvantages, one being that seeds can be sown too deep by mechanical planters. Fortunately, some lines of rice have evolved a mechanism to overcome this: mesocotyl elongation. The mesocotyl is a part of the rice seedling that elongates to push the seedling up allowing the plant to emerge from the soil and survive. See Figure 1 for more information on mesocotyl anatomy.

This life saving trait has been observed and studied, however, its specific molecular mechanisms are not completely understood. With a changing climate, methods for developing rice that can grow in adverse conditions are necessary. Assessing mesocotyl elongation is important because deep sowing is advantageous and the mesocotyl plays a crucial part in the success of deep sowing. Better defining the mechanisms behind mesocotyl elongation will maximize the benefits that come from deep sowing.

METHOD

- A mini core collection of 300 lines of rice from around the world were collected.
- 30 seeds from each of the 300 were grown in glass bottles in darkness for 10 days. See Figure 2
- Mesocotyl elongation was measured for each line and an average was calculated for each. See Figure 3
- 5 lines with the longest mesocotyl length, 5 lines with the most average mesocotyl length, and 5 with little to no mesocotyl length were selected and each was regrown.
- DNA was then isolated from each of the 15 sample lines.]
- Gel electrophoresis was run as well as a nanodrop spectrophotometery to quantitate the DNA and confirm its presence in the samples..
- Polymerase Chain Reaction (PCR) was run using RNA primers to isolate the GSK3 gene and amplify it.
- Gel electrophoresis was run to check if the PCR was successful.
- PCR results were sent for sequencing.
- Sequencing results were based on genomic DNA and required the introns to be spliced out to create cDNA.
- Nucleotide differences - also called single nucleotide polymorphisms (SNPs) - in the GSK3 gene between the lines were observed at positions 213, 390, 394, and 401. Based on the nucleotides present at these locations, lines were grouped into two haplotypes: Type A and Type B.
- The haplotypes were then analyzed to see if a correlation existed between the haplotype and mesocotyl length. More specifically, if Type B haplotype resulted in seedlings with longer mesocotyls.

RESULTS

Single Nucleotide Polymorphism(SNP) grouping into Two Haplotypes

Line Number	SNP1	SNP2	SNP3	SNP4	SNP GROUP
Reference	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
1	GCA (A213)	CAA (Q390)	GCC (A394)	AGG (R401)	Type B
2	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
3	GCA (A213)	CAA (Q390)	GCC (A394)	AGG (R401)	Type B
4	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
5	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
6	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
7	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
8	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
9	GCA (A213)	CAA (Q390)	GCC (A394)	AGG (R401)	Type B
10	GCA (A213)	CAA (Q390)	GCC (A394)	AGG (R401)	Type B
11	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
12	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
13	GCA (A213)	CAA (Q390)	GCC (A394)	AGG (R401)	Type B
14	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A
15	ACA (T213)	CGA (R390)	ACC (T394)	GGG (G401)	Type A

Line Number	Average Mesocotyl Length (cm)	Name	Country	Haplotype
1	1.734	KEERIPALA CHILL PADDY::IRGC 49790-1	India	Type B
2	0.1	XH211	Unknown	Type A
3	4.75	M 142::IRGC 35054-1	India	Type B
4	4.03	KALUT 139::IRGC 53670-1	Bangladesh	Type A
5	6.906	3210::IRGC 116950-1	Thailand	Type A
6	2.4099	Basmati 385	Pakistan	Type A
7	0.1	HP 3319-2WX-6-4-1-B::IRGC 117331-	Republic of Korea	Type A
8	1.3	Xiangwanxian 1	China	Type A
9	1.0866	NERICA 8	Cote d'Ivoire	Type B
10	2.917	EX FOILAEIN (NAPUTO)::IRGC 81675-1	Nigeria	Type B
11	0.1	HSINCHU AI CHIO CHIENG::IRGC 78182-1 12 94 0.03 SAN ZHAO QI::IRGC 72289-1	Taiwan	Type A
12	0.03	SAN ZHAO QI::IRGC 72289-1	China	Type A
13	6.1	UCP 122::IRGC 8794-1	Bangladesh	Type A
14	0.1	XH210	Unknown	Type A
15	3.98	BIR BAHADUR::IRGC 53889-1	India	Type A

FIGURES

Figure 1

Figure 2

Figure 3
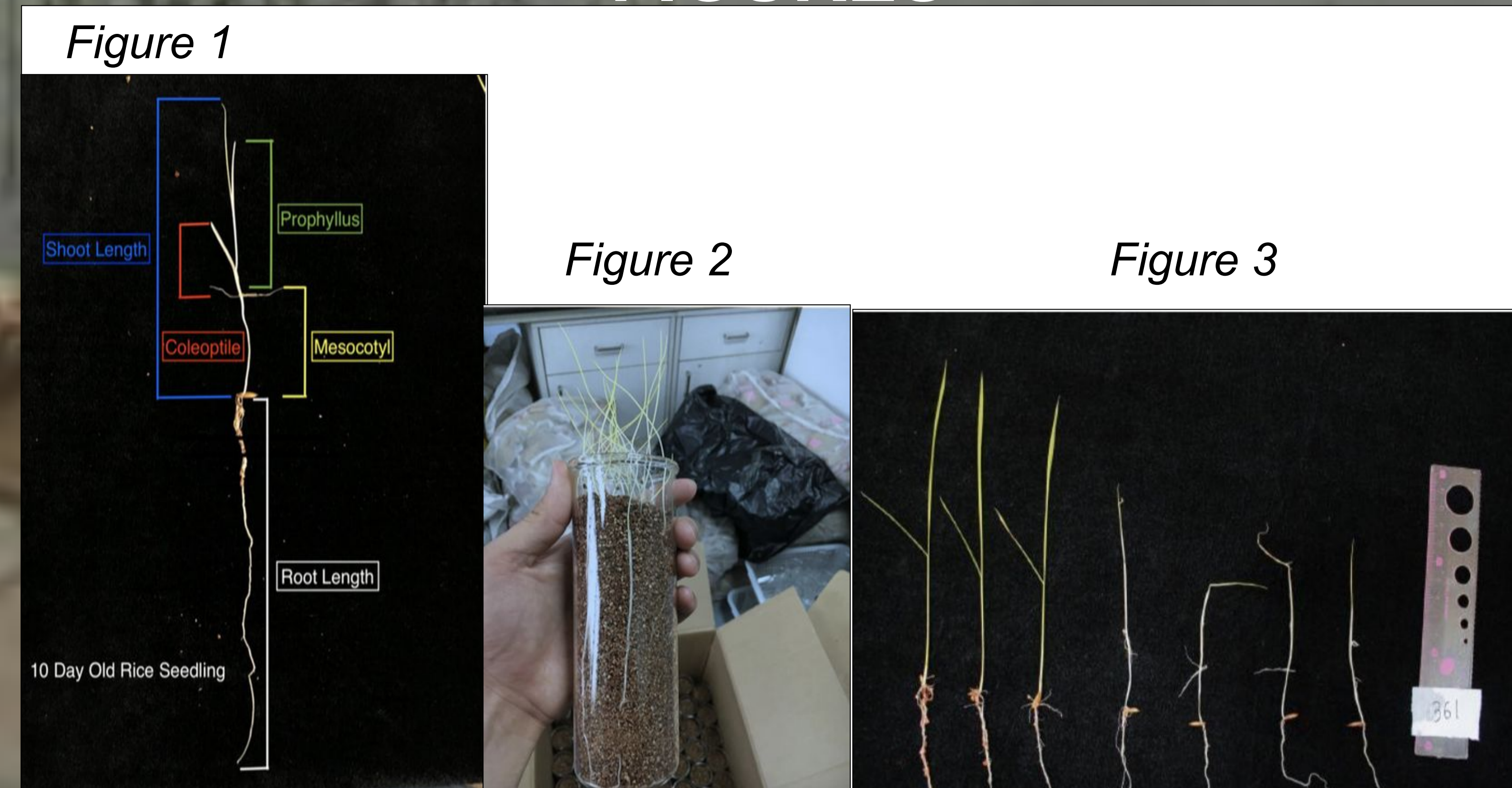
CONCLUSIONS

Our hypothesis was that SNPs observed in the GSK3 gene may lead to amino acid differences that account for a biologically significant change in protein shape or function causing longer mesocotyls. The results of this study support a partial trend, but not complete correlation of haplotype with mesocotyl length, suggesting that perhaps more than one genetic locus is involved in determination of mesocotyl length. Lines 1, 3, 9, 10, and 13 were classified as Type B, and none of them were part of the shortest mesoctyl length group. In fact, two were part of the longest group and three were part of the medium group.

To investigate the possibility that other genetic loci are involved in determining mesocotyl length the next step in this study would be to conduct a Genomic Wide Association Study (GWAS) to search for other possible genes responsible for this trait.

It is important to note that due to time constraints, this experiment was run only once. Regardless, it is generally rare to find phenotypes associated with a single gene. Most traits in nature are linked to more than one gene. According to this study and its results, GSK3 may not be the only gene responsible for the expression of mesocotyl elongation. Discovering the other, as yet unknown, gene(s) will be crucial in defining the mechanisms behind this life saving trait in rice seedlings. If the genes responsible for this trait as well as the associated SNPs are discovered, then farmers could select the most resilient lines, potentially yielding a higher success rate for seed germination, plant development, and most importantly, higher total crop yield. In conclusion, further defining the key mechanisms behind this trait has the potential to increase global rice production to ultimately help feed the ten billion people that are projected to inhabit our planet in 2050.

ACKNOWLEDGEMENTS