

Rice Phenotypic Implications of Tissue Culture-Induced Heritable Genomic Variation

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Abstract

Background: Plants that have been regenerated from tissue culture frequently exhibit somaclonal diversity. However, fundamental questions addressing the molecular traits, mutation rates, mutation spectra, and phenotypic significance of plant somatic variation have just lately been addressed. Additionally, these investigations have revealed outcomes that are wildly inconsistent between plant species and even within the same genotype [1].

Methodology/principal findings: An extensively selfed somaclonal line (TC-reg-2008) and its wild type (WT) donor underwent whole genome re-sequencing in order to study heritable genomic variation brought on by tissue culture in rice (cv. Hitomebore). Single nucleotide polymorphisms (SNPs), small-scale insertions/deletions (Indels), and transposable element mobilisation were all calculated (TEs). We evaluated the different genomic variation types' chromosomal distribution, explored the relationships between SNPs and Indels, and looked at the relationships between TE activity and cytosine methylation states [2]. We also conducted gene ontology (GO) analyses on genes carrying large-effect mutations and nonsynonymous mutations, and we assessed the effects of genomic changes on phenotypes under a variety of abiotic stimuli in addition to normal growth conditions. We discovered that rice had a large amount of heritable somaclonal genomic variation. Each of the 12 rice chromosomes has non-random genomic changes that had an impact on a significant number of functional genes. The conditional dependence of the genetic variants' phenotypic penetrance.

Conclusions/significance: Rice can be genetically modified by tissue culture in a way that differs significantly from natural environments, at least in terms of chromosomal distribution. Our studies have revealed fresh details about the somaclonal variance in rice's mutation rate and spectrum, as well as its chromosomal distribution pattern. Our findings also point to rice's great ability to channelize genetic variants under favourable conditions for maintaining phenotypic robustness, a capacity that, however, can be unleashed in response to certain abiotic challenges to produce varied phenotypes.

Keywords: Tissue culture; Single nucleotide polymorphisms; Somaclonal variance; Genetic variants

Introduction

It is well known that plants have the capacity to regenerate via in vitro tissue culture from totipotent, differentiated somatic cells. Somaclonal variation is the word used to describe a phenomena that has been linked to a number of genetic and epigenetic instabilities, some of which can result in heritable phenotypic variants [3]. Numerous instances of somaclonal variation involving a wide variety of plant species have been documented since the initial discovery of somaclonal variation in regenerated plants from sugarcane tissue culture. However, understanding of the molecular nature, mutation rate, and spectrum of somaclonal variation (nucleotide sequence alterations) has only recently been attained as a result of the revolutionary advances of modern analytical technologies, particularly next generation sequencing (NGS)-based genomic analysis [4]. In this study, we sequenced the entire genome of TC-reg-2008, a stabilised rice line that was developed from tissue culture of the Japanese rice cultivar Hitomebore and had been selfed for eight generations. From a genome-wide perspective, we compared the entire genome of TC-reg-2008 with the original wild-type (WT) donor at single-base resolution. We demonstrated that TC-reg-2008 had extensive and heritable genomic variations, including single nucleotide polymorphisms (SNPs), small-scale insertions and deletions (Indels), as well as transpositional reactivation of three TEs, despite the fact that this line under normal growing conditions showed no discernible phenotypic differences from its WT donor. Our findings have added to our understanding of the molecular characteristics underlying somaclonal variation in rice and demonstrated their

phenotypic canalization under normal condition [5].

Materials and Methods

Plant growth

East-West Seed graciously donated seeds of the *Artemisia annua* hybrid 1209. For surface sterilisation, the seeds were submerged in 10% commercial bleach (6.25% sodium hypochlorite; Chlorox) for ten minutes, followed by three rinses in sterile distilled water. After that, the seeds were put into GA7 culture jars (made by Magenta, Chicago, USA), each holding around 20 ml of MS basal media. Vitamins and MS salts made up the MS medium. Before agar was added and the medium was autoclaved for 20 minutes at 121°C and 21 pressure, the pH was adjusted to 5.7. The cultures were kept at 24°C 2°C in a growth environment with a 16-hour photoperiod (40 mol/m²/s) provided by cool-white fluorescent lighting.

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Plant tissue culture

Before the first genuine leaves appeared on 5-day-old seedlings, *Artemisia annua* explants were removed. Each seedling's two cotyledons and hypocotyl were detached and cultivated in separate Petri dishes (50 X 15 mm; Fisher Scientific, Canada), each containing three explants and around 10 ml of culture liquid. The basic media evaluated included 30 g/l sucrose, 7 g/l agar, 4.5 M 2,4-D, or either 11 M BA combined with 2.7 M NAA [6]. They were modified versions of those previously adjusted to generate and maintain undifferentiated tissue in *Artemisia annua*. 4.5 M 2,4-D medium mixed with 1, 10, or 100 M AIP, which was produced (SV ChemBioTech, Inc., Edmonton, AB) as previously described, was used to study the dosage response of AIP. For a more thorough investigation, 100 M AIP was either added or not added to media supplemented with 4.5 M 2,4-D, 11 M BA, or 2.7 M NAA. Agar was added to each medium, which had all been pH-adjusted to 5.7, and autoclaved for 20 minutes at 121°C and 21 pressure [7]. The cultures were kept at 24°C 2°C in complete darkness.

For the investigations on American elm (*Ulmus americana*) and sugar maple (*Acer saccharum*), callus was sourced from resources kept at the Gosling Research Institute for Plant Preservation. Both experiments used basal medium including MS salt and vitamins, 30 g/l sucrose, 7 g/l agar, 5 M BA (Sigma-Aldrich, Canada), and 1 M NAA to preserve callus that was initially obtained from mature trees (Sigma-Aldrich, Canada). The same media were used to place callus explants both with and without the addition of 1 mM AIP. Before checking for browning visually, cultures were cultivated for six weeks.

Sample preparation and extraction

Each *A. annua* culture plate's callus was weighed before being placed in a 15 ml centrifuge tube (Fisher Scientific, Canada), which was then quickly frozen in liquid nitrogen. Following that, samples were dried by lyophilization for at least 24 hours. A single observer rated the visual tissue browning of each sample on a hedonic scale from 0 to 10, with 0 denoting no discernible browning and 10 designating tissue that was dark brown or black. After being finely pulverised, samples were individually put into a 1.5 ml micro-centrifuge tube with about 10 mg (Fisher Scientific, Canada). Each tube received an aliquot of extraction solvent (1:1:1 water, methanol, and acetone), which was added to make the tissue to solvent ratio 1:10 [8]. After being vortexed, the tubes spent three hours in a sonicating water bath. The tubes were then taken out and centrifuged at 21.1g for 5 minutes. Each sample's supernatant was then put into a fresh micro-centrifuge tube.

Extract analysis

Gallic acid (Sigma-Aldrich, Canada) was used as the standard in a modified Folin-Ciocalteu test to calculate the total phenols. In a 96-well flat bottom microplate, 10 l aliquots of sample extracts, standards, or sample blanks were applied to each well. 100 l of the Folin and Ciocalteu phenol reagent were added to each well, and the plate was then incubated for 5 minutes before 80 l of aqueous 0.25 M Na₂CO₃ were added. After a further hour of dark incubation, the plate was read using a Synergy H1 microplate reader to measure the absorbances at 740 nm. All sample and standard readings were then adjusted using blanks [9].

As previously mentioned [34], absorbance at 340 nm was measured as a stand-in for evaluating tissue browning. To calculate the total phenolic content of the extracts, ferulic and chlorogenic acids (Sigma-Aldrich, Canada) were employed as standards. A 96-well flat bottom microplate was used, and aliquots of 10 l from each sample, standard

concentration, or sample blank were put to the wells. To each of the wells, 190 l more of the extraction solvent was poured. Using a Synergy H1 microplate reader, the absorbance from each well was measured. All sample and standard values were adjusted using the blanks. The spectral scan function was used to read the absorbance spectra of each sample, standard, and blank between 300 and 700 nm at 5 nm increments.

For all of the samples, the extracts' autofluorescent qualities were assessed together with possible standards such as ferulic acid, chlorogenic acid, cinnamic acid, and caffeic acid. In a 96 well black microplate, ten microlitre aliquots of each sample, standard, and blank were mixed with 190 l of extraction solvent. In the beginning, a Synergy H1 microplate reader was used to optimize the ideal excitation wavelength [10]. Based on prior research with phenolic-based blue-green autofluorescence of plants, a sample extract was used to test excitation wavelengths between 300 and 400 nm with a fixed emission wavelength of 460 nm. Using a fixed excitation wavelength of 360 nm from the previous optimization stage, the ideal emission wavelength was found by performing a spectral scan between 400 and 700 nm with 5 nm increments. This process was followed to create the fluorescence spectra for all samples, extracts, and blanks. Endpoint measurements were performed on each well at the optimal excitation/emission wavelengths of 360 nm and 450 nm, respectively. The average readings from the solvent blanks were used to correct each endpoint value and each spectral scan value [11].

Discussion

In this study, the whole genome re-sequencing of a stabilised rice line (TC-reg-2008) derived from tissue culture, followed by substantial selfing for eight successive generations, allowed for a detailed analysis of the genome-wide mutation rates, kinds, and spectra induced by tissue culture in rice. In several ways, our results differ significantly from the exclusion results published by other investigations. We found a mutation rate of 5.0×10^{-5} base substitutions per site, which is 21 and 29 times greater than those observed in regenerants of *Arabidopsis* and various genotypes of rice (Nipponbare), respectively. Additionally, 16,936 small-scale Indels were found in TC-reg-2008, which is significantly more than the number found in *A. thaliana* (only 21 were found), and Indels were not examined in the earlier work in the same species [12]. Additionally, seven mobilisation events by three retrotransposons were discovered and confirmed in this study, which differed from the two earlier investigations in rice, as one of them only revealed the activation of one TE (Tos17), while the other revealed the transposition of 17 TEs. In *Arabidopsis* regenerants, there was no TE activity. The distribution of the mutations on the chromosomes, which we found to be skewed vs. random in the tissue culture-induced mutations we found against those previously reported, is another important distinction. We demonstrated the non-random distribution of SNPs and small-scale Indels across each of the 12 rice chromosomes and the similar distribution pattern between SNPs and Indels. SNPs (single nucleotide substitutions) and Indels, in contrast, were discovered in the *Arabidopsis* study to map uniformly across the chromosomes. SNPs were found to be distributed at random across the rice chromosomes in the earlier rice study, however Indels were not examined. Both types of base substitution were found in TC-reg-2008. The pattern of base substitution can be transitions (Ts) or transversions (Tv). We found a rate of 2.37 transitions per transversion, which was distinct from the rates noted in rice and *Arabidopsis* regenerants. We do observe, however, that the within transition and transversion biases, i.e., G:C > A:T, were comparable to many previous reports on naturally

occurring mutations. This may be because all higher eukaryotes under study use the same highly conserved mechanism of deamination of methylated cytosines [13].

When considered as a whole, it is evident that the types, rates, and spectra of tissue culture-induced mutagenesis are highly variable and may differ depending on the species, genotypes, and tissue culture conditions, or may simply be accidental. Both previous studies and our data here showed that at least TE mobilisation occurs concurrently with DNA methylation dynamics, consistent with their repressive control by this epigenetic marker. This is contrary to our previous finding based on DNA marker analysis that genetic mutation is the major type of molecular changes associated with rice tissue culture. Given that tissue culture induced mutagenesis is known to occur stochastically, the participation of epigenetic processes supports the fortunate component of this process [14].

Conclusion

Prior research has discovered domestication-related rice areas that are genetically brittle and vulnerable to mutations in the presence of natural selection. Here, we demonstrated that there was minimal correlation between the genomic areas that were hyper-mutagenic in tissue culture and those that were hyper-mutable in the wild. This implies that natural mutation and tissue culture-induced mutagenesis may have different mutagenic pathways. Of fact, since the somaclonal line (TC-reg-2008) we utilised has only experienced eight generations without deliberate selection, we cannot completely rule out the notion that long-term selection was what made the difference. However, we believe that further research is necessary to elucidate any novel potential applications of tissue culture in crop development.

Acknowledgement

None

Conflict of Interest

None

References

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