Approaches to improving wheat quality by targeting local induced lesions in genomes

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Introduction
Wheat is a staple element in the flour responsible for the curation of many dishes such as pasta, bread, etc. Because of the widespread consumption of this cereal, it is imperative to uphold the quality of this important element to ensure proper nutrient distribution.

Objectives
- Asparagine synthesis in durum wheat leads to the direct production of acrylamide, a toxic carcinogen to the human body. (Curtis & Halford 2016) This project aims to limit, and even abolish, acrylamide synthesis in wheat products. This hexaploid species focuses on three genomes: A, B, and D.
- Research has displayed that high-molecular-weight subunits have a direct correlation to the quality of gluten structure as well as functionality of the wheat. (Shewry et al. 1991) This project focuses on targeting the Gy12 molecular subunit, a HMW subunit that is essential to dough quality. (Fig. 1)

What is TILLING?
TILLING, targeting local induced lesions in genomes, is a reverse genetic technique that combines the use of mutagenesis and high throughput molecular techniques.

Methodology
- Bread wheat is a hexaploid species, and grains that were used in this study were germinated using EMS-TILLING giving the A, B, and D genomes the label, ASN2. ASN2 contains mutations that reduce asparagine expression.
  - DNA was extracted from grains belonging to these three mutants and then were amplified using PCR techniques. Gel electrophoresis was used to confirm the presence of these mutations.
  
  In order to send the HMW subunits to sequencing, cloning was done on proteins containing the Gy12 sequence in order to amplify this particular part. During the procedure, ECOR1 restriction enzymes were used to target the desired base pairs.

  Grains from durum wheat were germinated using knockout genes aiming to reduce prolamin proteins through TILLING had the label of BPBF A or B.
  - The identification of these knockout genes were solidified through an acid-polyacrylamide gel for gliadins and an SDS gel for glutenin.
  - The purpose of the acid-polyacrylamide gel was to ensure that the gliadin bands were absent (the protein responsible for gluten components) and the bands identifying glutenin were intense. Glutenin is responsible for wheat quality.
  - The two gels are used because of the different solubility properties of glutenin and gliadin. The acid-polyacrylamide gel is used to separate proteins, and the SDS gel is used to separate based off of the protein molecular weights.

Results
- After the amplification of DNA samples from the ASN2 samples, it was confirmed that the A and D genome were the only grains that contained the mutant genes. The B genome proved to show unsuccessful bands.
  - The acid-polyacrylamide gel of the gliadin proteins showed successful mutant strains by being absent in the gel. (Fig. 2)

Results Continued
- The glutenin bands recovered from the SDS gel showed intense bands indicating that glutenin proteins were present.
  - Cloning for the Gy12 protein was repeated twice as the linear plasmid solution was not effective (PGEM). The process was then repeated and had successful results, so the base pairs were sent to sequencing.

Conclusion
- When data is retrieved from sequencing regarding ASN2 (A and D genomes), the homoalleles will be crossed to create a pure mutant species. The germination of the B genome will be redone with extracted DNA from new grains.
- After sequencing will be done on the prolamin binding factors, the grains with the mutant homoalleles will be crossed.
- The HMW subunit, Gy12, will be used to testcross these mutant homoalleles, and more studies will be conducted on how to incorporate this HMW into daily sustenance for consumers.

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