AGRICULTURAL RESEARCH FOUNDATION
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TITLE: Fast-Track Development of Potato Clones with Pure Amylopectin Starch Used in the Paper, Textile and Food Industries by Using Induced Mutation.

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Summary:
The potato is the world's fourth most important food plant – after rice, wheat and maize. But that's not all: the tuber's starch content also makes the modest spud an extremely versatile and sustainable raw material. The USA is the largest producer of starch in the world, at approximately 30 million tons a year. In the USA, maize is almost the sole raw material (only 2% of its production is derived from wheat and potatoes). Of the total world starch production, including Europe, about 82% is derived from maize, this is followed by wheat (around 6%), and the root crops, potatoes (6%) and cassava (6%).
Potatoes are becoming more and more important as renewable raw materials for the starch industry. The starch produced in potatoes, however, isn't in an ideal form. It's composed of a mixture of two different kinds of starch: amylose and amylopectin. These two kinds of starch have very different properties. Amylopectin, making up 80 percent of the starch content in potatoes, consists of large, highly-branched molecules. Amylopectin makes starch water soluble and gives it its characteristic stickiness. It is very useful in the food, paper, and chemical industries as paste, glue or as a lubricant. Amylose is made up of long, chain-like molecules and is used predominantly in the production of films and foils. Both of these kinds of starch are useful for human nutrition. But for the processing industry, a mixture of different starches is a problem. Industry must separate the two kinds of starch using expensive processes that take a toll on the environment. In order to reduce costs and environmental load the potato starch industry is seeking for amylase free potatoes.
In recent years there have been many product developments based on starch, including biodegradable eco-plastic. Foam starch-based packaging chips are now widespread. And the use of bioplastics is already established in the production of carrier bags that can also be used for

collecting compostable waste, and in the production of trays for foodstuffs (2). Starch (mainly potato starch) is used as an oil-drilling fluid. Most petroleum producing countries do not produce starch but rely on imports. There is a great potential market in the world petroleum sector particularly in the Middle East. Amylopectin is highly coveted component in the industry. This is why plant breeders are working hard to develop potatoes that produce only one type of starch. Right now, emphasis has been placed upon developing potatoes containing only amylopectin, due to its diverse applications. Classical breeding methods have not yet been able to provide an amylose-free potato that has acceptable yield and resistance to pests and diseases. In potato, the importance of granule-bound starch synthase gene (GBSSI gene) function is realized through a number of published reports covering both basic and applied research during more than past two decades (1) (3). Some of the research activities include generation and characterization of the potato mutants for the production of amylose-free starch where the mutation was located in the GBSSI gene. The Genetic engineering (Antisense-Strategy), offers a targeted approach to suppressing the production of amylose, which has disadvantages in terms of public acceptance and high costs for regulatory approval of genetically modified organisms. Recently, an amylose-free potato has been successfully developed by using chemical and gamma irradiation. Using molecular biological methods plants that show the desired genetic changes can be rapidly detected.

Potato improvement through strain (sub-clonal, intraclonal, or line) selection due to natural mutation within a variety has been practiced for years. In 2012 more than 40% of USA seed potato production derived from natural mutant selections from established varieties. Thanks to OSU ARF competitive grant the Oregon breeding program has integrated induced mutation to generate new clonal variants in established potato varieties. The development of potato variety with only amylopectin starch will have a great advantage for Oregon potato industry.

The main purpose of the current study was to induce mutations in six elite potato varieties to generate clones with pure amylopectin starch for industrial use. The proposed methodology integrates molecular markers for early screening and tissue culture techniques for fast generation of desired potato clones. Gamma irradiation at was used to induce mutants in vitro grown shoot tips

Objectives:

1. Induce mutation in selected established potato varieties by using gamma irradiation
2. Screening putative mutants for amylose & amylopectin
3. Molecular marker screening for GBSSI gene mutation
4. Produce tubers in greenhouse for further increase & evaluation
5. Planting the selected mutants with high amylopectin in the field for evaluation

Materials & Methods:

The most popular established potato varieties (Three processing varieties: Russet Burbank, Ranger Russets & GemStar Russet) and (Three fresh market potatoes: Russet Norkotah ,Yukon Gold and Dark Red Norland) were used for this study. In vitro grown shoot-tips (1-5mm) were used for Gamma irradiation treatments. For gamma irradiation three hundred shoot-tips were excised from each variety and were cultured in sterile solid Murashige and Skoog (MS; *SigmaChemicalCo*.,St. Louis, USA) media in plastic Petri plates . The pH of the media was adjusted to 5.8 before autoclaving (for 20 min at 121 °C). The cultures were maintained at 25 ± 1 °C, and a 16-h photoperiod with irradiance of 51 µmol m-2s-1. Hundred shoot tips (25 shoot-tips per plate) in four plates per treatment were irradiated at 10 (1000rad), 20 (2000Rad), and 40 (4000 Rad) grays doses of γ- rays for creating genetic variability. Irradiated shoot tips and the control were transferred into new MS media. After four weeks the regenerated plantlets were cut into regular MS media for further evaluation. The experiments were set up in a completely randomized design with four replicates with 25 plants per plate. Analysis of variance was performed and significant differences among treatment means were calculated by LSD test at $P < 0.05$.

Isolation of variants

In vitro microtubers were produced from all putative mutants and the controls in MS media supplemented with 4mg/l Kinetin and 90g/l sucrose. Nodal cutting (100 ca) from each clone were cultured in Petri plates (10 per plate). The cultures were maintained in dark conditions for 8 weeks, at 20ºC. The harvested micro tubers were used to for early screening of amylose and amylopectin. A portion of micro tubers were cut and sprayed with a 2% iodine solution (2g KI and 0.2 g I2 in 100 mL of distilled water). Waxy type will stain reddish brown, and non-waxy type, will stain dark blue. We used the pictures of the starch grains to classify the levels of amylopectin. To further confirm the results microtubers from each clone were squeezed though potato ricer and starch was extracted for analysis. Amylose content were determined by extracting the starch with perchloric acid followed by determination of the absorption at 618nm and 550nm after staining with Lugol's solution (3).

DNA extractions, PCR& Sequencing

Genomic DNA was extracted from 30-50mg of young leaf tissue using DNeasy QIAGEN kit. Polymerase chain reactions (PCR) was carried out at varying annealing temperatures using GBSSI gene-specific primers. PCR was performed in a total volume of 20µl containing final concentration of 1X PCR buffer, 1.5 mM $MgCl_2$, 2% mixed solution of sucrose in cresol red (2% (w/v) of sucrose, 0.1 mM of cresol red), 0.1 mM dNTPs, 0.5 µM of each primer, 0.03U Fermentas Dream *Taq* DNA polymerase and total 40 ng of genomic DNA. PCR conditions were varied with the primers used in the study. PCR products were visualized using agarose gels.

Analysis of PCR amplification products and subsequent sequencing were provide an overview of GBSSI allelic composition of putative mutants (1).

Greenhouse increase & field studies

In the second year potato clones selected with high amylopectin (amylose-free) were increased to produce seedling tubers in greenhouse. Selected amylose-free mutants will be grown in the field in randomized block design with four replications. Phenotypic data on plant emergence, plant height, flower color, maturity, tuber yield, tuber size, shape, fry color etc. will be collected and statistically analyzed by using statistical software.

Enzymatic measurement of the amylose and amylopectin

The amylose and amylopectin contents of matured tubers grown in the field will be determined using a Megazyme (K-AMYL 07/11: Megazyme International Ireland Ltd. (Bray, Ireland).

Statistical analysis: All experiments were performed in replicated trials as indicated above. The analysis of variance of the data was performed using the SAS statistical software (For Windows V9.1, SAS Institute Inc., Cary, NC, USA). The difference among the mean values were processed by Duncan's Multiple Range Test, and significance will be defined at $P<0.05$.

Major Accomplishments:

- High level of amylopectin producing potato clones were identified in three potato varieties (Russet Burbank, Russet Norkotah, and GemStar Russet)
- We established fast protocol to use Gamma irradiation to generate mutants of potatoes
- We have also established non-GMO genome editing techniques to improve potato varieties by inducing specific mutations
- We are now increasing in the greenhouse to putative high amylopectin producing clones for field evaluations.

BENEFITS & IMPACT:

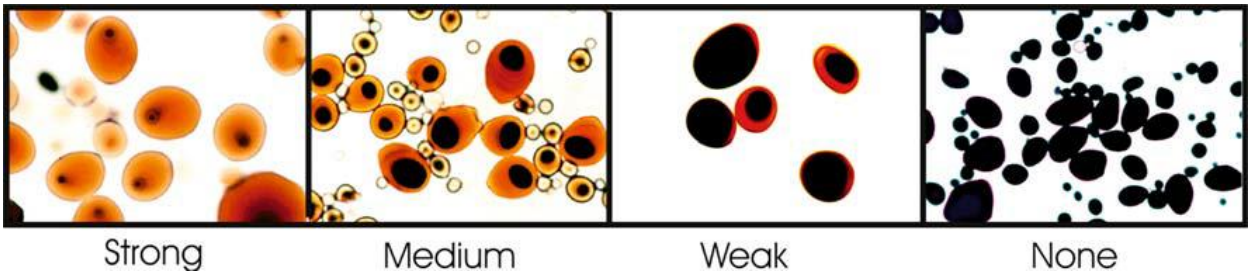
The development of potato variety with only amylopectin starch will have a great advantage for Oregon potato industry in particular & at large in national level. Amylopectin is highly coveted component in the industry. There is a great potential market for biodegradable eco-plastic and in the world petroleum sector.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

The Oregon variety development & breeding program received grants in the amount of more than $90,000 to support all our activities from Oregon Potato Commission.

FUTURE FUNDING POSSIBILITIES:

We are planning to submit proposal to **THE CONSORTIUM FOR PLANT BIOTECHNOLOGY RESEARCH, INC.**



Classification of potato clones with different levels of amylopectin in percent.

Clones	Strong	Medium	Weak	None
Russet Burbank	3	2	11	84
Russet Norkotah	14	4	0	82
GemStar Russet	11	6	0	83

Yukon Gold	0	0	0	100
Ranger Russets	0	0	0	100