# Enriching Peanut with Essential Amino Acids

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## ABSTRACT

❖ Introduction

Plants are the main source of dietary proteins consumed by humans and livestock. However, plant proteins are generally considered as incomplete proteins due to their deficiency in several essential amino acids. Peanut (Arachis hypogaea L.) is a nutrient-dense legume and a major source of plant protein, with about 24% proteins. Peanut flour is used in formulated foods given as therapeutic foods to aid in famine relief. However, peanut seed proteins are deficient in essential amino acids (EAA) including methionine, threonine, isoleucine and tryptophan. Recent advances in biotechnology offer the prospects of improving the nutritional profile of food crops through genetic engineering, thereby producing high-value products.

Objective

The objective of this investigation is to enhance the essential amino acid content of peanut, in order to improve its nutritional quality.

Methodology

An Artificial Storage Protein (ASPx) gene encoding a storage protein rich in essential amino acids (methionine, lysine, tryptophan, threonine, isoleucine, leucine, valine and phenylalanine) was introduced into peanut via Agrobacterium-mediated gene transfer.

Results and discussion

Ten (10) independent kanamycin resistant plants were regenerated from transformed peanut hypocotyl cells. The phenotypic characteristics were similar to control non transformed plants. Molecular analysis using PCR and Southern hybridization indicate the stable integration of the ASPx gene in the peanut genome. The presence of the ASPx protein in peanut crude extracts was confirmed by multiple reaction monitoring (MRM). These results indicate that the nutritive quality of peanut can be enhanced via biofortification.

**Keywords: Biofortification, Essential Amino Acid, Storage protein, Peanut**

## METHODOLOGY

Transformation cassette

The plasmid pDK612 was obtained by subcloning the artificial storage protein (ASPx) gene into the binary vector pLAU2.

Transformation of peanut seeds

pDK612 was mobilized into Agrobacterium strain EHA 105 by electroporation. Peanut hypocotyls from 6 day-old seedlings were transformed according to Dodo *et al*, 2008 protocol.

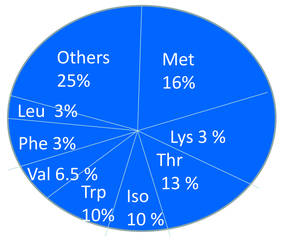
Screening of transgenic plants and seeds

➢Genomic DNA extracted using Qiagen kit.

➢q-PCR was run in a Lightcycler 480, Roche. Primers and probe were designed with the Primer Express Software target the ASPx gene.

➢Mass spectrometry based method (multiple reaction monitoring) of low molecular weight proteins from the crude protein extracts of peanut seeds was used to assess the presence of the ASPx protein.

Acids amino composition of the ASPx



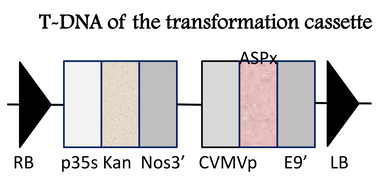




**Fig 1: Amino acids content in the ASPx protein and map of the T-DNA used for the genetic transformation**

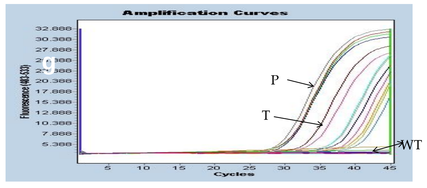
## RESULTS

### 1. Production of transgenic plants and seeds


a: peanut seeds
b: peanut embryo
c: peanut embryos in culture
d: 6-day-old seedling
e: inoculation with *A. tumefaciens* suspension
f: hypocotyls in KanR selection medium (SM)
g: shoots in SM
h: shoot in rooting medium
i: plants in green house
J: transgenic seeds

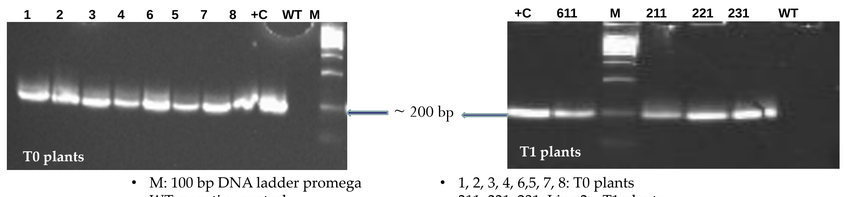
**Fig 2: Production of transgenic plants and seeds**

### 2. Insertion of transgene plants: qPCR




•P: Positive control: plasmid pDK30
•WT: Negative control: Genomic DNA from wild type
•T: Samples: Genomic DNA from putative transgenic plants

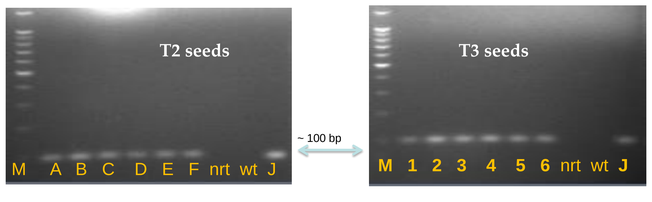
**Fig 3: Quantitative RT-PCR (TaqMan qPCR) to detect the presence of the ASPx gene in plants**




- M: 100 bp DNA ladder promega
- WT: negative control
- ASPx: positive control
- 1, 2, 3, 4, 6,5, 7, 8: T0 plants
- 211, 221, 231: Line 2 – T1 plants
- 661: line 6 – T1 plant

**Fig 4: Polyacrylamide Gel Electrophoresis of Taqman-PCR products**

### 3. Expression of transgene plants at mRNA level: RT-PCR




M: 100 bp DNA ladder (promega)
A-F: transgenic T2 seeds
Wt: wild type
nrt: non reverse transcriptase negative control
J: positive control
1, 2, 3, 4, 5, 6: T3 seeds plants

**Fig 5: Gel Electrophoresis of reverse transcriptase RT-PCR products**

### 4. Expression of ASPx at the protein level

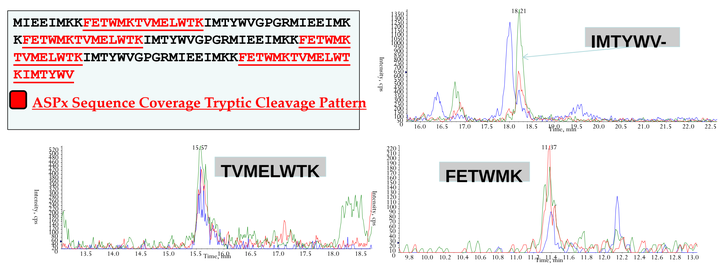



**Fig 6: Detection of the presence of ASPx protein by MRM**

## RESULTS

### 6. Phenotypic characteristics




WT: wild type
WT/TC: wild type grown via tissue culture
Trsg: transgenic

**Fig 7: Phenotypic characteristics comparison of transgenic *vs.* wild type**

## DISCUSSION

• qPCR analysis shows the integration of the ASPX gene into peanut genome for To plants and the inheritance of the transgene for the second generation of plants (T1 plants).

• Reverse transcriptase RT-PCR shows that the ASPx gene was expressed at the transcript level in the transgenic seeds.

• Multiple Reaction Monitoring demonstrates the presemve of the ASPx protein in the transgenic seeds *vs.* the wild type peanut seeds.

•Data indicates that the protein content of peanut may be improved Via genetic transformation.

•Further analysis (nutritional analysis) will help estimate the increase in essentials amino acids.

## Abbreviations

ASPx : Artificial Storage Protein
MRM: Multiple Reaction Monitoring
RM: Rooting medium
RT-PCR : Real-Time Polymerase Chain Reaction

## REFERENCES

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2. Konan NK, Viquez OM and Dodo H, 2004. Towards the development of a hypoallergenic peanut through genetic transformation. Applied Biotechnology, Food Science and Policy 1 (3):159-168.

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