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COMPARISON OF NUTRITIONAL VALUE OF TRANSGENIC PEANUT EXPRESSING *bar* AND *rcg*3 GENES WITH NON-TRANSGENIC COUNTERPARTS

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Abstract

The transgenic peanut plants expressing *bar* and *rcg*3 genes were subjected to assessment of any change in nutritional value of the crop at various locations. The protein and fat contents of transgenic lines were compared with the non-transgenic parent varieties. Protein content in the transgenic lines was higher as compared to that in non-transgenic counterparts and differences among locations for fat and protein content were significant. No differences among fatty acids were recorded for genes, events and locations. Irrespective of small differences, all the values were in range described for this crop and transgenic lines appeared to be substantially equivalent to non-transgenic parent varieties.

Key words: Transgenic peanut, *bar* and *rcg*3 gene, Nutritional value comparison.

Introduction

The risks related with transgenic crops are a result of complex interactions between the specific transgenic event, the history of organism, and the nature of the ecosystem in which that crop is going to be released. Introduction of a new gene in the crop is supposed to change the genetic makeup and ultimately the nutritional composition of that specific crop. Therefore, the public is now becoming very conscious regarding the use of transgenic crops especially used as food and feed.

While studying the impact assessment of the transgenic biotechnology, the idea of familiarity is normally associated with substantial equivalence. The principle behind this concept is: "if a new food derived from the transgenic crop is found to be substantially equivalent in composition and nutritional characteristics to an existing non transgenic counterpart, it can be regarded as being as safe as the conventional food" (Anon., 2003). In order to assess the potential effect or secondary effect of the genetic insertion on plant biochemistry, analytes, including a certain number of important nutrients, antinutrients, and toxicants used for crops, were selected to speculate this effect (Kier & Petrick, 2008). The objective behind that is to not set absolute levels of food safety, but relative levels; so that it can be proved that no environmental risk will arise due to cultivation of transgenic plant under projected production conditions.

Peanut is an important oilseed crop normally used as roasted nuts. It has oil content of more than 50% depending upon the species and environmental condition in the growing area. It is also a rich source of protein to be used as food or feed (25-30% proteins). Potential Yield of peanut is limited by a number of biotic and abiotic factors. Weeds and fungal diseases are very common in the growing area of peanut causing significant reduction in the yield. Conventional techniques did not offer a real solution to the problem.

The technique of genetic engineering may offer the solution of the problem by incorporation of herbicide resistance genes in peanut so that herbicide can be applied safely. Lack of host resistance against early leaf spot disease (tikka disease) is also another factor to be addressed. Insertion of gene against fungal diseases is also known to be useful in increasing the farm yield. Genetic transformation technology has a potential for development of peanut lines resistant to variety of herbicide and pathogens, threatening the peanut health and ultimately yield (Jonnala 2005). The insertion of foreign genes in the commonly cultivated peanut varieties is supposed to alter the nutritional composition of the grains; therefore the comparison of the transgenic crop along with non transgenic parent is very important to assess the nutritional composition.

Ridley *et al*., (2002) studied the compositional equivalence in forage and grain of herbicide tolerant transgenic maize with its non transgenic counterpart and other commercially grown varieties. Al though some differences observed were statistically significant, but the values recorded lies within the range of commercially grown varieties and the values found in literature. Many scientist reported that the variations in composition of plant are may be due to differences in analytic methods used (Escher *et al*., 2000; Saxena & Stotzky, 2001) age of plant material at the sampling and analysis time and the transformation method used in the transgenic varieties under observation. Similar results were also reported by Appenzeller *et al*., (2009) that the nutritional value of transgenic maize was similar to non transgenic maize.

The objective of the present study was to investigate the nutritional composition of transgenic peanut expressing *bar* and *rcg*3 gene along with the non transgenic counterparts at major peanut growing areas.

Material and Methods

Herbicide resistant *bar* gene and leaf spot resistant rice chitinase (*rcg*3) were grown at Groundnut Research Station (GRS), Attock, Barani Agricultural Research Institute (BARI), Chakwal and National Agricultural Research Centre (NARC), Islamabad along with their non transgenic counterparts. At maturity seeds were collected, air dried and packed for further analysis.

Protein analysis: Protein analysis of Peanut seeds was carried out using the method described by Anon., (1984). In this method 1 g of well grinded, homogenized sample was added in 5 g of digestion mixture in a micro-kjeldhal's digestion flask. About 30 ml of conc. sulphuric acid was added and flask was placed for digestion for 3-4 hrs, until the solution become clear (green colour). The flask was then cooled down and the contents were transferred in 250 ml flask, and final volume was made with water. Then 10 ml of this solution was placed in micro-kjeldhal distillation apparatus followed by boiling of 10 ml 40% NaOH through steam. As a result ammonia was liberated, condensed and collected in a beaker having 10 ml of 2% boric acid solution. When boric acid colour changed from pink to yellow titrated against 0.1N H_2SO_4 solution till light pink end point.

Crude fat: For the extraction of crude fat weighed 2g of sample in a filter paper, made a thimble properly and placed it in the extractor of the Soxhelet's Apparatus. A receiver was adjusted with the apparatus containing hexane at 80°C in water bath, heated it about for 3 hours with the rate of 80-90drops/ min. Then removed the thimble or filter paper, and again adjusted the apparatus at 60-90°C to recover the access solvent until about 2-3 ml remained in the receiver. After that transferred the contents of receiver in pre- weighed Petri-dish, washed with petroleum ether 2-3 times and collected the aliquot in Petri dish. Placed the dish in an oven (60°C) until evaporation of solvent, cooled it in the desiccators and weighed it again (Anon., 1984).

Fatty acid analysis: FAMEs analysis was used for analysis of fatty acids. The FAMEs analysis was performed using a Clarus 500 (Perkin Elmer, USA) auto-system gas chromatograph with flame ionization detector (GC-FID). A fused silica capillary column was used. An aliquot (2.0µl) of the FAME extract was injected in split (13.5:1) mode at 250°C. The carrier gas used was nitrogen with 1.6 ml/m flow. The GC oven temperature programme was 50°C for 1 min raised to 150°C at 15°Cmin^{-1}, raised to 175°C at 2°C min^{-1} (held for 2 min) and finally raised to 220°C at 2°C min^{-1} (held for 5 min). Total GC runtime was 49.35 min. The FID detector was set at 275°C. Acquisition of data and reprocessing were performed using a Total *Chrom Workstation* version 6.3.1. Identification of fatty acids was performed by comparison with the fatty acid methyl ester standard (68A) and was quantified using peak area percentage as a ratio to total area of all methyl esters.

Statistical analysis: The data obtained was subjected to statistical analysis by using Analysis of variance (ANOVA) under completely randomized design. The statistical analysis was carried out using the Sigma plot version 10 Software. A confidence level of 95% for the F-distribution was selected and treatment mean obtained was compared by LSD at 5% level of significance.

Results

Protein percentage in the peanut seeds having *bar* and *rcg*3 was not significantly different from each other, however the differences among the transgenic and non transgenic seeds were significant ($p<0.05$). Protein content of both varieties was higher in the transgenic seeds as compared to non transgenic seeds (Fig. 1). The seeds having *bar* gene contain 23 % higher protein than its non transgenic control and the seeds having *rcg*3 have 8% higher protein in the crops harvested from NARC. Whereas in case of crop harvested from GRS, the transgenic having *bar* gene gave similar yield and having *rcg*3 gave 7% higher protein content. The transgenic seeds contain 7% lower protein than non transgenic seeds having *bar* gene and similar protein in seeds having *rcg*3 gene at BARI. Furthermore protein content did not differ significantly at different locations. The interaction among genes and transgenic and non transgenic seeds and also between genes and locations was not significant as there were no statistical differences in the protein content at all experimental locations irrespective of genes.

The fat content was not significantly different in the seeds of peanut having different genes and among transgenic and non transgenic events ($p<0.05$). Percent fat in the seeds was statistically different among the three experimental locations that indicate that climatic and soil conditions at different locations played a major role on the fat percentage in the seeds. The peanut seeds harvested from BARI had 3% and 10% more fat than the seeds from GRS and NARC respectively (Fig. 2).

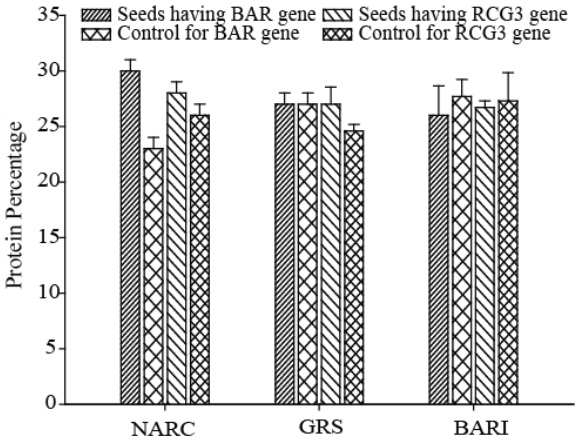

Fig. 1. Protein content of transgenic and non transgenic seeds at different locations.

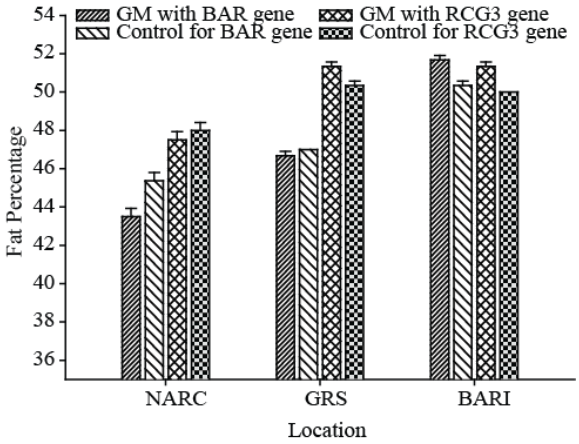

Fig. 2. Fat content of transgenic and non transgenic seeds at different locations.

Table 1. Fatty acid composition of peanut seeds having *bar* and *rcg*3 genes along with their respective control harvested from BARI, Chakwal.

Genes	Events	Palmatic acid	Oleic acid	Stearic acid	Arachidic acid	Linoleic acid	Eicosanoic acid	Behenic acid	Tetracosanoic acid
Bar	Transgenic	7.7 ±0.01	44±1	2.4±0.1	1.2±0.1	25±2	2.2±0.1	2.4±0.1	1.3±0.5
	Non-Transgenic	7.7±0.01	44±1	2.3±0.1	1.2±0.1	26±2	2.2±0.1	2.4±0.1	1.3±0.5
*rcg*3	Transgenic	7.6±0.01	44±2	2.4±0.1	1.2±0.1	22±2	2.1±0.1	2.3±0.1	1.7±0.5
	Non-Transgenic	7.7±0.01	45±1	2.4±0.1	1.2±0.1	23±1	2.2±0.1	2.2±0.1	1.3±0.5

Table 2. Fatty acid composition of peanut seeds having *bar* and *rcg*3 genes along with their respective control harvested from GRS, Attock.

Genes	Events	Palmatic acid	Oleic acid	Stearic acid	Arachidic acid	Linoleic acid	Eicosanoic acid	Behenic acid	Tetracosanoic acid
Bar	Transgenic	7.7±.01	44±1.1	2.4±0.1	1.2±0.1	24±1.5	2.2±0.2	2.4±0.1	1.6±0.5
	Non-Transgenic	7.7±.01	44±1.5	2.4±0.1	1.2±0.1	25±2.0	2.1±0	2.3±0.1	1.3±0.5
*rcg*3	Transgenic	7.7±.01	44±1.0	2.3±0.1	1.2±0.1	25±1.5	2.1±0.1	2.2±.1	1.7±0.5
	Non-Transgenic	7.7±.02	43±0	2.3±0.1	1.2±0.1	23±1.5	2.3±0.1	2.2±.1	1.3±0.5

Table 3. Fatty acid composition of peanut seeds having *bar* and *rcg*3 genes along with their respective control harvested from NARC, Islamabad.

Genes	Events	Palmatic acid	Oleic acid	Stearic acid	Arachidic acid	Linoleic acid	Eicosanoic acid	Behenic acid	Tetracosanoic acid
Bar	Transgenic	7.7±.01	43±1.2	2.4.±0.5	1.1±0.1	24±2	2.4±0.1	2.1±0.1	1.3±0.5
	Non-Transgenic	7.6±.01	44±1.2	2.3±0.5	1.2±0.1	23±2	2.2±0.1	2.2±0.1	1.3±0.5
*rcg*3	Transgenic	7.6±.01	43±2.3	2.4±.05	1.3±1.3	23±2	2.2±0.1	2.2±0.2	1.3±0.5
	Non-Transgenic	7.6 ±.01	44±1.5	2.3±0.5	1.2±0	24±2	2.3±0.1	2.3±0.2	1±0

The crop having *bar* gene contained 52% fat at GRS, 51% at BARI and 43% at NARC, while the crop having *rcg*3 gene contained 47% fat at GRS and 51% BARI and 47% at NARC. The percentage of fat in the transgenic seeds was 2.0% and 2.6% higher than non transgenics at BARI and NARC respectively, while no statistical differences were recorded at GRS. There were also significant differences when the fat percentage was pooled over locations, genes and transgenic and non transgenic seeds. Highest fat percentage was observed in transgenic seeds at GRS and BARI while lower fat percentage was recorded in the plants having both genes at NARC irrespective of the transformation events. Although some significant differences in the fat percentage were observed but all were in the range described for the test crop.

Non-significant differences among the genes, events and locations were recorded in almost all fatty acids except Eicosanoi acid and Tetracosa (Tables 1-3). Transgenic seeds have 18% higher content of Tetracosanoic acid than the non transgenic seeds irrespective of the inserted gene.

Discussion

Irrespective of the fact that some significant differences and interactions for nutritional parameters were recorded but all the values lies in the range, reported in the literature for peanut seeds (Tayyab *et al*., 2011 and Grosso *et al*., 2000). The observed differences are attributed to the complex interaction of genotype, environmental conditions and the fertility status of the soil under experimentation.

Protein is very important nutrient and its presence in the seed can also be a toxin, allergen or antinutrient. As an important dietary factor its presence is essential component of human diet (Delaney *et al*., 2008). In the present study the increase in protein content was observed due to genetic modification and the differences among locations were also significant. The reason for the higher protein content in the seeds harvested from NARC might be attributed to the higher nitrogen content in the soil (data not shown) as the increase in nitrogen content of the soil is already reported to increase the protein content of the seeds (Akbari *et al*., 2011).

The increase in the protein content due to genetic modification is also observed in the earlier studies. Jonnala *et al*., (2005) reported that transgenic peanut having rice chitinase gene had total protein contents between 25 to 28% similar to parent variety, Okrun (Jonnala, 2004). Similar results were reported by Kosieradzka *et al*., (2001) and Osama *et al.,* (2007).

Several other researchers (Teshima *et al*., 2002) also proved that there were no remarkable compositional differences in amino acids, and protein content of transgenic and non transgenic crops. Hongju *et al*., (2006) also conducted a study to compare nutritional value of GM conventional green peppers. The components (moisture, energy, fat, protein, fiber, carbohydrates and ash) and minerals were analyzed in the seeds and pericarps. The study demonstrates that the differences were not significant in the nutritional content between the GM and the conventional crop. No differences in the protein and amino acid content of transgenic and non transgenic rice (having *bar* and *cry*1Ac1) were recorded for protein and amino acid content by Park *et al*., (2013).

Fat accumulation in Peanut is influenced by number of factors like temperature, fertilization, moisture availability, and their interaction. Higher percentage of fats at BARI may be attributed to higher temperature at BARI during the flowering period. Demurin *et al*., (2000) also found an increase in oil percentage due to increase in temperature during flowering to maturity in Sunflower and Maize. They also reported that 1^{o}C rise in

temperature increased oil content by 1% in Sunflower. Similarly, Kaleem & Hassan (2010) observed variation in oil content in different circles of sunflower head which was mainly driven by temperature.

Qin *et al*., (2012) compared the fatty acid content of AVP1- expressing peanut plant with its wild relative. They observed the similar content of major fatty acids. Differences among the minor fatty acids (stearic acid, behenic acid and lignoceric acid) were also detected among transgenic and wild type plants: however these differences were also observed among wild type and segregated non transgenic lines. The authors concluded that introduction of AVP1 gene in peanut did not affected the oil and fatty acid content of the seed. In another study Mei *et al*., (2011) inserted chitanase gene in maize plant. Significantly higher content of all the six kinds of fatty acids were detected in transgenic maize as compare to those in non-transgenic seeds of maize.

Little increases of protein and fatty acid in transgenic lines may be attributed to improved plant health as a consequence of control of fungal diseases.

Conclusion

The insertion of *bar* and *rcg* 3 gene had no affect on the protein and fat content of the peanut seeds and transgenic peanut seeds are substantially equivalent to their non transgenic counterparts. Irrespective of small variations all values were in range described for this crop and genetic engineering had no negative impact on the dietary value of the harvested grains.

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