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Studies on Protein Composition of Pigeon Pea [*Cajanus Cajan* (L.) Millspaugh] Treated With Sodium Azide and Gamma Radiation

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Abstract: *The aim of this study was to determine the protein composition of pigeon pea Cajnus cajan (L) millspaugh treated with sodium azide and gamma radiation. This was performed by exposing the seeds of landraces pigeon pea to gamma rays at Centre for Radiotherapy and Oncology Department, ABUTH, Shika, Zaria at doses of 0(control), 50, 100, 150 and 200Gy. These seeds were further treated with sodium azide (NaN_3) concentrations at 0.00, 0.01, 0.02, 0.03 and 0.04% SA. The analysis of protein content was the determination of the amount of reduced Nitrogen present in food substance, i.e., its $–NH_2$ and $=NH_2$. The proximate analyses were carried out as recommended by the Association of Official Analytical Chemists (AOAC, 1990) using the kjeldahl method. The highest crude protein (CP) percentage value was obtained from 150Gy + 0.03%SA (25.1 %), followed by that due to 100Gy + 0.01%SA (24.7 %), 100Gy + 0.04%SA (24.4 %), 100Gy + 0.03%SA (23.7 %) and 150Gy + 0.00%SA (23.6 %). It is, therefore, concluded that the two mutagens significantly affected the pigeon pea plant composition.*

Keywords: *Protein composition, crude protein, nitrogen, sodium azide, gamma radiation, pigeon pea, Cajanus cajan.*

I. Introduction

Pigeon pea belongs to Family Fabaceae, sub family Papilionaceae, tribe Phaseoleae, sub-tribe Cajanae, genus *Cajanus*, species *cajan* (L.) Millspaugh, which also contain soybean (Glycine max (L) and Field bean (Phaseolus vulgaris (L), an often cross-pollinated diploid 2n = 2x = 22, 44 or 66 chromosomes (Varshney et al., 2012). Pigeon pea is an erect perennial legume shrub often grown as an annual, reaching 91- 366cm in height. Pigeon pea is heat-tolerant, prefers hot moist conditions. It grows in temperature between 18^0C and 30°C and the optimum rainfall required for pigeon pea is 600-1000 mm/year. A rain at flowering time has very adverse effect on the seed yield (Edwards, 1981).

Pigeon pea does well in low fertility soils with reasonable water-holding capacity and pH 5-7 is favourable for its growth. Pigeon pea does not tolerate shallow soils or water logging. It tolerates a wide range of soils, from sandy to heavy black clays. It is sensitive to salt spray and high salinity. It is a short day plant, flowering is triggered by short days, whilst with long days plant grows vegetatively and the leaves have three leaflets that are green and pubescent above and silvery greyish – green with longer hairs on the underside. The flowers are yellow with red to reddish-brown lines or red outside (Centre for New Crops and Plants Products, 2002).

Pigeon pea improves the physical, chemical, and biological properties of the soil; it functions as a mini-nitrogen factory (Okaka et al., 2002). The plant is rich in protein and fats, making it an ideal supplement to traditional cereal, banana or tuber-based diets of most Africans which are generally protein deficient. Protein content of commonly grown plant ranges between 18 – 26% (John, 2005). The seed is made up of 85% cotyledon, 14% seed coat, and about 1% embryo and it is a rich source of carbohydrates, minerals and vitamins. It's CHO content ranges between 51.4 – 58.8%, CF ranges between 1.2 – 8.1% and lipid ranges between 0.6 – 3.8% (Singh et al., 1990). The plant seed is also a good source of dietary minerals such as Calcium, Phosphorus, Magnesium, Iron, Sulphur and Potassium and water soluble vitamins especially thiamine, riboflavin, niacin. It contains more minerals, ten times more fats, five times more vitamin A and three times more vitamin C than cereal grains. It also provides firewood and income for poor small scale farmers (Saxena, 2000).

The objective of this study is to determine the effects of sodium azide and gamma rays on the protein composition of the pigeon pea.

Research Hypothesis: The mutagens have no significant effect on the protein content of pigeon pea plant.

II. Materials And Methods

Source of Research Materials

The seeds of pigeon pea (*Cajanus cajan* (L.) Millsp.) was obtained from local farmers in Ankpa Local Government Area, latitude 7^o 38'E and longitude, 7^o 22'N (163m elevation above sea level), Kogi state, Nigeria (Garmin eTrex Venture HC Handheld GPS). The Gamma radiation source (Cirus Cobalt 60 Teletherapy) used

for seed treatment was located at the Radiology and Oncology Department, Ahmadu Bello University Teaching Hospital Shika, Zaria. The Sodium azide (SA) used for this research work was manufactured by KEM Light Laboratories P.V.T. Ltd.

Study Area

This research was conducted both in the laboratory and in the Biological Garden of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria (Longitude 07° 39'E and latitude 11° 09'N, 2148 above sea level), Nigeria. (Garmin eTrex Venture HC Handheld GPS). Samaru lies in the Northern Guinea savannah agro-ecological zone of Nigeria with mean annual rainfall of about 1100m. Rainfall in this region is essentially between May and September and dry season between October and April. Hottest months of the year are March and April and the region is with a mean daily temperature of 27°C. The coldest months are November – January (Osuhor et al., 2004).

Treatments

Exposure of Seeds to Gamma Radiation

Uniform healthy dry seeds of *Cajanus cajan* (L.) Millspaugh were exposed to different doses of gamma rays (control 0, 50, 100, 150 and 200Gy), derived from Cobalt-60 (^{60}CO) source with a measured dose rate of 124.5Gy/min which lasted for 8hrs 52mins at the Oncology Department, Ahmadu Bello University Teaching Hospital, Zaria.

Seeds Treated with Sodium Azide

The radiated healthy and dry (10-12% moisture) seeds of *Cajanus cajan* (L.) Millspaugh were pre-soaked in a phosphate buffer (pH 3.0) to maintain the osmotic content of the cell for six (6) hours and later subjected to the four (4) concentrations (0.01%, 0.02%, 0.03% and 0.04%) of sodium azide [SA] (NaN_3) solutions at room temperature (25°C) for six (6) hours. The seeds were washed thoroughly to remove the residual amount of mutagens and sown immediately.

Collection of Soil Samples

Top soil was collected from uncultivated land within the Botanical Garden in Ahmadu Bello University, Zaria. A sample of the top soil was air dried and taken to the Department of Soil Science, Institute for Agricultural Research, Zaria for physico-chemical analysis.

Pot Preparation and Seed Planting

Top soil was used to fill six hundred and fifty (650) polythene bags and four (4) treated seeds were sown inside each polythene bag during rainy season. The polythene bags were arranged in a complete randomized design (CRD).

Determination of Nitrogen and Crude Protein

The proximate analyses were carried out as recommended by the Association of Official Analytical Chemists (AOAC, 1990) using the kjeldahl method. The analysis of protein content was determination of the amount of reduced Nitrogen present in food substance, i.e., its $-NH_2$ and $=NH_2$. There are major compounds in food containing Nitrogen but minor nitrogenous ingredients of food include Amino acids, Purines, Ammonium salts and Vitamin B_1. So Nitrogen is used as an index of protein termed 'crude protein' as distinct from true protein. Proteins determination was carried out in three stages, as follows:

a. Digestion: Two grams of sample was weighed and placed into a 50ml digestion – flask and the Kjeldahl mixture which acts as a digestion catalyst was added. The flask containing the sample mixture was heated gently at an inclined angle in a Kjeldahl digestion rack until frothing subsided. It was then boiled until the solution became colourless. Heating of the mixture released the Nitrogen in the various samples which was then converted to ammonia with the concentrated Sulphuric acid. It was later allowed to cool. The sample was transferred to a 100ml volumetric flask and diluted with distilled water to the mark. It was then mixed thoroughly. The mixture was further allowed to cool before distillation. A blank containing only the Sulphuric acid and catalyst was also heated

$$\text{Sample (N)} + \text{Conc. } H_2SO_4 \longrightarrow (NH_4)_2SO_4\,(\text{N} - \text{free})$$

$$C \xrightarrow[\text{Oxidation}]{} CO_2$$

b. Distillation: A known aliquot (10ml) was transferred to the distillation apparatus and then introduced to the sample chamber 10ml of 40% sodium hydroxide was added to the sample addition tunnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing 10ml 2% boric acid solution into which 4 drops of bromocresol green and 2 drops of methyl red indicator had been put. Distillation was continued until the pink colour turned greenish.

$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O + 2NH_3$

$NH_3 + H_2O \longrightarrow NH_4OH$ (ammonium hydroxide)

$H_3BO_3 + 3NH_4OH \longrightarrow (NH_4)_3BO_3 + H_2O$

c. Titration: Back titration method was employed, i.e., the ammonia reacts with the Boric acid in the receiving flask and the amount of excess acid is determined by titration with Hydrochloric acid.

$(NH_4)_3BO_3 + 3HCl \longrightarrow NH_4Cl + H_3BO_3$

The percentage total Nitrogen was calculated and crude protein was estimated by multiplying the percentage Nitrogen with standard conversion factor 6.25. (i.e. % crude protein (cp) = 6.25 x N

$$\% \text{ N} = \frac{V_1 - V_0 \text{ x M x } 14 \text{ x } 100 \text{ x } 100}{0.2 \text{ x } 1000 \text{ x } 10 \text{ x } 1}$$

V_o = Vol. of Hydrochloric acid require for blank
V_1 = Vol. of the Hydrochloric acid required for 10ml sample solution
M = Molarity of acid (0.1M)
14 = atomic weight of N
100 = total volume of digest
100 = % conversion
10 = Volume of distillate
0.2 = amount of sample taken in gram

Note: protein contains 16% N_2. This makes the general conversion factor to be 6.25.

Statistical Analysis of Data

The data obtained from the parameters yield parameters were subjected to statistical analysis to assess the extent of induced variations using the analysis of variance (ANOVA) to establish if there was any significant difference between the means of the doses/concentrations of the mutagens using Science Analysis Software (SAS) Version 9.0 2004.

Duncan's Multiple Range Test (DMRT) was used to separate and compare means where significance difference was observed using Science Analysis Software (SAS) Version 9.0 2004.

III. Results

The range of values obtained for crude protein is presented in Table 1 below. The highest crude protein value was obtained from R3A3 (150Gy + 0.03% SA)(25.1 %) was significantly higher than that due to all the other treatments ($P \leq 0.05$). This was followed by that due to R2A1 (100Gy + 0.01 % SA)(24.7 %), R2A4 (100Gy + 0.04 % SA)(24.4 %), R2A3 (100Gy + 0.03 % SA)(23.7 %), R1A2 (50Gy + 0.02% SA)(24.0 %) and R3A0 (150Gy + 0.00 % SA)(23.6 %) treatments. The least crude protein obtained from treatment R0A1 (0Gy + 0.01% SA)(18.6%) was only similar to that due to R3A4 (150Gy + 0.04 % SA)(18.7 %) treatment (Table 1).

Table 1: Effect of Gamma Radiation and Sodium Azide on Some Yield Parameters of Pigeon Pea

Mutagen	CP (%)
R0A0	18.9^{p}
R0A1	18.6^{q}
R0A2	19.8^{o}
R0A3	21.3^{g}
R0A4	20.0^{klm}
R1A0	19.4^{p}
R1A1	20.1^{klm}
R1A2	24.0^{d}
R1A3	21.8^{f}
R1A4	21.3^{g}
R2A0	20.3^{jk}
R2A1	24.7^{b}
R2A2	20.0^{lmo}
R2A3	23.7^{de}
R2A4	24.4^{c}
R3A0	23.6^{e}
R3A1	20.2^{jkl}

R3A2	20.6^{hi}
R3A3	25.1^{a}
R3A4	18.7^{pq}
R4A0	20.4^{ij}
R4A1	19.9^{lmo}
R4A2	20.4^{ij}
R4A3	20.0^{lmo}
R4A4	20.7^{h}
SE±	0.09

Means followed by the same letters along column are not significantly different (P >0.05)
Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.0% SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,
CP = Crude Protein

IV. Discussion

Generally, there is a high performance in most of the mutagenic treatments over the Control treatment, with 18.9% crude protein. The higher seed protein content in plants with mutagenic treatments could be due to the genetic improvement brought about by the mutagens. Similar result was obtained by Shinde (2007) in Pigeon pea, Sagabe (2008) and Urdbean and Tambe (2009) in Soybean. It has been reported that protein production is directly linked with the quality of seeds; the better the quality of seed is, the more the production of protein. Therefore, in the case of increased protein content the seed quality also increased. It may also be due to interactions between genes and the environment (Singh et al., 1990). According to Khan et al. (2000), the improvement made in protein content through genetic manipulation could make it a good source of dietary protein to man. Protein is involved in chromosome organization needed for chromatic separation and segregation in plant.

V. Conclusion

This study revealed relatively High protein content in the pigeon pea mutants by exposed to maximum doses and concentrations of sodium azide. The mutants isolated in the present investigation would be of great utility in cultivation of pigeon pea.

VI. Recommendation

Further work should be employed using doses/concentrations of gamma rays and sodium azide to determine the effects in inducing resistance against diseases and cooking time in pigeon pea.

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