Asian Research Journal of Agriculture



14(4): 82-88, 2021; Article no.ARJA.72653 ISSN: 2456-561X

# Agrophysiological Characterization of Maize (Zea mays) Plants from EV 8728 Seeds Irradiated to Gamma Radiation

Ayolié Koutoua<sup>1\*</sup>, Beugré Manéhonon Martine<sup>1</sup>, Kanga Ahou Nadia<sup>1</sup>, N'guessan Affoué Sylvie Rachelle<sup>1</sup>, Kanga N'guessan Martial<sup>2</sup>, Kouadio Kouamé Léonard<sup>1</sup>, Kouadio Akéssé Blaise<sup>1</sup>, Koné Sié Kissomanbien<sup>1</sup> and Kouadio Yatty Justin<sup>1</sup>

<sup>1</sup>Laboratory for Improving Agricultural Production, Faculty of Agroforestry, University Jean Lorougnon Guédé, BP 150 Daloa, Côte d'Ivoire. <sup>2</sup>Laboratory of Plant Physiology, Faculty of Biosciences, University Félix Houphouët Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.

## Authors' contributions

This work was carried out in collaboration among all authors. Authors AK, BMM and KYJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KKL, KSK and KAB managed the analyses of the study. Author NASR managed the literature searches. All authors read and approved the final manuscript.

# Article Information

DOI: 10.9734/ARJA/2021/v14i430141 <u>Editor(s):</u> (1) Dr. M. Yuvaraj, Adhiparasakthi Agricultural College, India. <u>Reviewers:</u> (1) Ramiro Cruz Durán, Universidad Nacional Autónoma de México, México. (2) Sami Ali Metwally, India. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/72653</u>

> Received 13 June2021 Accepted 26 August 2021 Published 05 November 2021

**Original Research Article** 

# ABSTRACT

Maize (*Zea mays* L.) is a tropical annual herbaceous plant of the Poaceae family. It is a cereal cultivated under very varied conditions ranging from tropical to temperate climates. However, the accentuated climate change causes abiotic and biotic stresses reducing the development and the production of the plants. To improve these plants, it is important to understand the mechanisms involved in these stresses. Two doses of gamma irradiation (200 and 300 grays) were applied to the seeds of a variety (EV8728) of maize grown on an experimental plot at the Jean Lorougnon Guédé University (UJLoG). Thus, the morphological, physiological and biochemical behaviors of

the plants were evaluated. The results showed that gamma radiation significantly reduces morphological parameters. However, this reduction is accentuated at 300 grays. At the physiological and biochemical level, the radiation reduced the chlorophyll a, b and total (t) contents and the carotenoid content. Additionally, the contents of proline, total protein and total sugar increased with 200 grays and decreased for 300 grays. However, the phenolic compounds content increases with the irradiation dose.

Keywords: Maize; irradiation; gamma radiation.

## **1. INTRODUCTION**

Côte d'Ivoire is a country whose development is based on agriculture. Agricultural production accounts for 33% of the gross domestic product and 75% of export earnings. Agriculture employs nearly 67% of the working population in Côte d'Ivoire [1]. However, this agriculture is primarily based on industrial crops (wood, coffee, cocoa, cotton, rubber, oil palm, cashew, pineapple etc.) to the detriment of food crops which nevertheless are part of our daily regarding food. Among these crops, we can cite maize, which plays a very important socio-economic role.

Maize (Zea mays L.) is a tropical annual herbaceous plant of the Poaceae family. It is a cereal cultivated under very varied conditions ranging from tropical to temperate climates. In the world, Maize is the first cereal (41%) cultivated in terms of quantity and area, ahead of wheat 40% and rice 9% with world production estimated at 1091 million tons during the 2019-2020 campaign. The world maize market is driven by the United States, Brazil, Argentina, Ukraine and secondarily by South Africa [2]. In Africa, since 2018, its production is estimated at around 79 million tons for an area of 38,673,230 hectares [3]. The central role of maize as a staple food in sub-Saharan Africa is comparable to that of rice or wheat in Asia. Maize makes up almost half of calorie and protein intakes in Eastern and Southern Africa, and one-fifth in Western Africa [4]. In Côte d'Ivoire, maize occupies a prominent place in agricultural activities, in human and animal nutrition. National production increased from 1,025,000 tons in 2017 for an area of 523,538 ha to 1,006,000 tons for a total area of 473,143 ha [3]. Nearly 50% of this production is located in the Savannas region located in the north of the country [5]. According to FAO production estimates and compared to the situation in neighboring countries, the Ivorian maize sector appears to be growing relatively slowly. However, despite the increase in maize production in the world, its cultivation is faced with many problems. These problems are related

not only to soil degradation but especially to climate change. Recent studies suggest that major product production has declined since 1980 due to global warming [6]. It is estimated that under current global warming trends in sub-Saharan Africa, major cereal production could decline by up to 20% by mid-century [7]. In order to contribute to finding a sustainable solution to climatic variability and induced fertility loss, the IAEA-funded maize project has initiated a research program on the development of maize varieties adapted to the soil conditions of northern Côte d'Ivoire using induced mutation techniques. The general objective of this study is to evaluate the effect of gamma radiation on the agro physiological parameters of a variety of maize grown in Côte d'Ivoire and mainly in the department of Daloa.

## 2. MATERIALS AND METHODS

## 2.1 Plant Material

The plant material consists of seeds of the local improved maize variety EV8728 irradiated with gamma radiation at doses of 200 and 300 grays in Seibersdorf, Austria.

## 2.2 Methods

## 2.2.1 Study site

Research was conducted for four months (June-September) at the University Jean Lorougnon Guédé, located in the department of Daloa. The city is located in the region of Haut Sassandra in the Center-West of Côte d'Ivoire between 6° and 7° North latitude and 7° and 8° West Longitude. This June-September period corresponds to the rainy period and the second maize production cycle in this area. The soil substrate of Daloa belongs to the old Precambrian basement composed of granites, migmatites and granitogneis. These soils, leached and deep (20 m), are due to the abundant rainfall and the rapid alteration of the rocks. The soils of the region are mostly ferralitic. The experimental field of our trial

was on a flat ground and presented a vegetation dominated by grasses and interspersed with some woody species.

#### 2.2.2 Experimental device

This study was carried out using a completely randomized block experimental design with two replicates. Each block included three elementary plots sown according to the different doses. Each block was 40 m x 20 m, or 800 m<sup>2</sup>. The distance between the two blocks was 2 m. The seeding was done at 0.8 m between the rows and 0.4 m on the row (between pits). Each block consisted of several lines at a rate of 9 lines for the 200 and 300 treatments as well as for the control, for a total of 27 lines per block. Seeding was done at a rate of 2 grains per poquet for each dose. Each block consisted of several lines at a rate of 9 lines for the 200 and 300 treatments as well as for the control, for a total of 27 lines per block. Seeding was done at a rate of 2 grains per poquet for each dose.

#### 2.2.3 Observation and measurements

The effect of irradiation was evaluated by vegetative. physiological and biochemical parameters. The vegetative parameters measured were: stem diameter determined with a caliper, plant height determined with a carpenter's meter and the number of panicle spikelets determined by counting. All these measurements were taken 67 days after sowing on more than 1000 maize plants. For physiological and biochemical parameters, we extracted and measured chlorophyll pigments and carotenoids according to the method of Lichtenthaler [8], total phenols according to the method of Singh [9], proteins according to the method of Bradford [10], proline according to the method of Dreir and Goring [11] and total sugars according to the method of Dubois et al. The second leaf from the top of the stem was used for the dosage. Samples were taken after the plant had released all its pollen.

#### 2.2.4 Statistical analysis

The data were statistically analyzed with STATISTICA software, version 7.1. Analysis of variation (ANOVA) was used to test statistical significance, and significant differences between the means were calculated using Tukey's HSD test at P < 0.05.

## 3. RESULTS

## 3.1 Effect of Gamma Radiation on Vegetative Parameters

In order to better understand the mechanisms used by the plants to morphologically reveal possible mutations, several agro-morphological variables have been taken into account. Thus, the analysis of all three vegetative parameters studied showed a significant effect (p < 0.001) (neck diameter and stem height) and (p < 0.01) (number of spikelets) for the different irradiation doses used (Table 1).

## 3.1.1 Effect on collar diameter

Stalk thickness growth of the studied maize variety is influenced by the different applied gamma irradiation doses (Table 1). The diameter decreases as the irradiation dose increases. This reduction is 9.13% and 16.14% respectively for the 200 and 300 grays doses compared to the control.

## 3.1.2 Effect on stem height

Expression of plant height growth is under the control of gamma irradiation doses. Indeed, the application of gamma irradiation on maize seeds reduces the height growth of the plant (Table 1). This reduction increases as the irradiation dose increases. Thus, the reduction rate is 21.7% at the level of 200 grays and 25.41% at the level of 300 grays compared to the control.

Table 1. Influence of gamma radiation on collar diameter, stalk height, and number of spikelets
of maize plants

	Collar diameter(mm)	Stalk height (cm)	Number of spikelets
Control	16,313 ± 0,874 <sup>a</sup>	141,165 ± 14,892 <sup>a</sup>	16,000 ± 1,414 <sup>a</sup>
200 grays	14,825 ± 0,758 <sup>b</sup>	110,540 ± 4,080 <sup>b</sup>	16,167 ± 1,602 <sup>a</sup>
300 grays	13,681 ± 1,122 <sup>b</sup>	105,303 ± 7,503 <sup>b</sup>	14,000 ± 1,414 <sup>b</sup>
P	0,001	0,001	0,040
F	12,078	22,918	3,985

Values followed by the same letter are not significantly different (5% Tukey test)

#### 3.1.3 Effect on the number of spikelets

The results for the average number of spikelets per male flower of the studied maize variety are recorded in Table 1. The average number of spikelets at the 200 grays rate is approximately equal to that of the control, but at the 300 grays rate, there is a 12.5% reduction compared to the control.

## 3.2 Effect of Gamma Radiation on Physiological and Biochemical Parameters

Statistical analysis of the results of these parameters showed a very highly significant effect (p< 0.001) (chlorophylls b, carotenoids, phenolics, total protein, proline, and total sugars) and a significant effect (p< 0.01) (chlorophylls a and total) for the different doses of gamma irradiation used (Tables 2 and 3).

## 3.2.1 Effect on chlorophyll pigments

Gamma radiation has an influence on chlorophyll synthesis in leaves (Table 2). Indeed, at high doses, gamma radiation decreases the content of chlorophyll a, b and total. In these pigments, the reduction rate is 17% (chla), 15% (chlb) and 17% (chlt) at the 300 grays dose compared to the control. This rate decreases as the irradiation dose decreases. Thus, at 200 grays, the rate is 6%, 0%, and 5% for chlorophyll (a), (b), and total, respectively. The chlorophyll content decreases with increasing irradiation dose.

## 3.2.2 Effect on carotenoids

The results for carotenoid content in the corn leaves studied are reported in Table 2. The carotenoid content of the leaves is reduced by 21% at 300 grays, but at 200 grays the content is approximately equal to that of the control.

# 3.2.3 Effect on the content of phenolic compounds

The variation in phenolic compound content of leaves from different irradiation doses is shown in Table 3. Indeed, under the effect of gamma radiation, the content of phenolic compounds accumulates as the irradiation dose increases. This very highly significant accumulation (p < 0.001) is a function of the irradiation dose. Thus, the accumulation rate is 168% and 193% respectively in the 200 and 300 grays doses compared to the control.

#### 3.2.4 Effect on proline content

The variation of proline content in leaves from plants irradiated with different doses of gamma radiation is shown in Table 3. The proline content increases at low doses and decreases at high doses of irradiation. Indeed, the proline accumulation rate is 52% at 200 grays versus 39% at 300 grays compared to the control.

## 3.2.5 Effect on total protein content

The total protein content decreases when a high dose of gamma irradiation is applied (Table 3). Indeed, the reduction is seen at the 300 grays dose with a rate of 10%, but at 200 grays there is rather a slight increase of 3% compared to the control.

#### 3.2.6 Effect on total sugar content

Under gamma irradiation, total sugars accumulate in the leaves of the studied corn variety (Table 3). However, this accumulation is more pronounced at the 200 grays dose, representing a 79% accumulation rate compared to the control. On the other hand, although total sugar accumulation at 300 grays is higher than the control, it is still lower (52%).

Table 2. Influence of gamma radiation on	leaf pigment content of maize variety
--	---------------------------------------

Leaf pigment content (µg /mL)						
	Chlorophyll <sup>a</sup> (chl a)	Chlorophyll <sup>b</sup> (chl b)	Total chlorophyll (chl t)	Carotenoid		
Control	1,00 ± 0,129 <sup>a</sup>	0,263 ± 0,029 <sup>a</sup>	1,263 ± 0,157 <sup>a</sup>	0,325 ± 0,052 <sup>a</sup>		
200 grays	0,940 ± 0,078 <sup>ab</sup>	0,265 ± 0,023 <sup>a</sup>	1,208 ± 0,096 <sup>ab</sup>	0,324 ± 0,028 <sup>a</sup>		
300 grays	0,828 ± 0,069 <sup>b</sup>	0,222 ± 0,010 <sup>b</sup>	1,050 ± 0,076 <sup>b</sup>	0,259 ± 0,020 <sup>b</sup>		
P	0,021	0,006	0,015	0,009		
F	5,034	7,248	5,560	6,526		

Values followed by the same letter are not significantly different (5% Tukey test)

	Phenolic compound (mg/g MF)	Proline (mM/g MF)	Protein (mg/g MF)	Total sugars (mg/g MF)	
Control	90,948 ± 5,484 <sup>a</sup>	0,172 ± 0,016 ª	4,497 ± 0,258 <sup>a</sup>	76,617 ± 7,829 <sup>a</sup>	
200 grays	152,882 ± 12,430 <sup>b</sup>	0,262 ± 0,015 <sup>b</sup>	4,642 ± 0,118 <sup>a</sup>	136,967 ± 11,557 <sup>b</sup>	
300 grays	175,003 ± 15,101 <sup>c</sup>	0,106 ± 0,009 <sup>c</sup>	4,030 ± 0,212 <sup>b</sup>	116,067 ± 9,529 <sup>c</sup>	
Ρ	0,001	0,001	0,001	0,001	
F	82,816	187,926	14,684	59,177	
	Values followed by the same letter are not significantly different (5% Tukey test)				

Table 3. Influence of gamma radiation on phenolics, proline, protein, and total sugars content of maize variety leaves

#### 4. DISCUSSION

#### 4.1 Vegetative Parameters

The results showed that gamma irradiation of maize seeds has adverse effects on plant growth and development. Our results also revealed that increasing the dose increases the depressive effect on growth, high doses reduce growth. Our results are in agreement with Sergio et al. [12] who explained that gamma radiation significantly reduces growth parameters with increasing doses in Triticum turgidum. According to Ilyas and Naz [13], the maximum height of the plant is achieved at lower doses of gamma irradiation. This height is reduced as the doses become higher and higher in curcuminoid and essential oil plants of turmeric (Curcuma longa). In general, increasing doses of irradiation have detrimental effects on the growth of the plant. This is in agreement with the work of Saiful et al. [14], who observes that higher doses of gamma radiation are detrimental to the growth of young grape plants. According to [15], the changes caused by irradiation may be due to inhibition of seed germination. The germination rate of barley seeds irradiated at higher doses significantly decreases with aging compared to the control and seeds irradiated at lower doses. This could be explained by the fact that stress blocks the mechanism of cell division [16]. The effect of gamma rays is dose dependent, low doses would cause less damage compared to high doses of irradiation. These effects affect more the phenotype of the plant, including the biochemical components. At low doses of irradiation, the defense mechanism of the plant is activated. This allows the plant to resist against any induced mutations. Regardless of the dose used, the gamma ray creates crop mutants [16].

## 4.2 Physiological and Biochemical Parameters

The physiological approach in this study showed that chlorophylls a, b, total and carotenoids contents are negatively influenced by gamma irradiation. Gamma radiation has very highly significant effects in chlorophylls b compared to chlorophylls a. On the other hand, according to Tumuluri and Syed [17], chlorophyll content decreases upon treatment with UV-A, but rather chlorophyll a is more affected compared to chlorophyll b in four crops namely red range, peanut, wheat and ragi. This reveals that UV-A has adverse effects on photosynthesis and ultimately leads to the reduction of plant productivity. The work of He et al. [18] revealed that treatment of Tradescantia fluminensis with carbon ion irradiation significantly decreases chlorophyll a, b, total and carotenoid contents. On the other hand, Abd El-Rahman et al. [19] mentioned that the highest content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in dried mint leaves was observed in the high dose irradiated samples.

Biochemical results showed that there is an accumulation of proline, total sugars, and phenolic compounds under the effect of gamma irradiation doses. Indeed, under the effect of gamma radiation, the content of phenolic compounds increases even at high doses. Our results are similar to those of Fereshteh et al. [20] who by studying the effect of gamma radiation in date samples showed that phenolic compound content increases at high doses. On the other hand, Fabiana et al. [21] revealed that irradiation of baby cores with gamma radiation reduces the phenolic compound content at high radiation doses. The increase in phenolic compounds allows the plant to protect itself against biotic and abiotic agents. The total protein content decreases with increasing

irradiation dose. This was consistent with the results of Fatehi et al. [22] performed on pistachio. These results show that the upregulation of some physiological characteristics and growth of rice seedlings from gamma radiation treatment can be used to control abiotic stresses such as drought and salt [23]. At the level of total sugars, there is an accumulation after gamma irradiation. Hanafy et al. [24] showed that gamma radiation increases the content of total sugars in the shoots of damsissa plants. The results obtained in our work indicated proline gamma irradiation causes that accumulation in the plant. Ashrafijou et al. [25] revealed that proline content significantly increases with laser beam irradiation on oilseed rape. This accumulation of proline contributes to stress resistance through the maintenance of cell turgor in the species allowing water entry into the plant. Its role is necessary for osmotic adjustment [26] On the other hand, a high accumulation of this amino acid is a sign of metabolic disturbance [27].

## 5. CONCLUSION

Gamma irradiation had both a detrimental and a beneficial effect on the plants of the maize variety on all vegetative, physiological and biochemical parameters studied. The degree of sensitivity or tolerance depends on the dose of irradiation applied. Indeed, the increase of the irradiation dose reduces the growth of the corn variety. The chlorophyll a, b and total chlorophyll contents are very sensitive parameters that represent indicators of the tolerance level of the maize variety (EV8728). The action of gamma radiation resulted in levels of total phenolics, proline and sugar that correlated with the irradiation dose. The mechanisms studied in this work allowed to establish differences in the behavior of maize plants from irradiated seeds. These are the maintenance of a good osmoregulation (proline) and a good functioning of the photosynthetic apparatus.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

# REFERENCES

- 1. CNRA. Cultures annuelles. 2013;46.
- Frédéric H. Production mondiale de maïs. En ligne disponible en ligne sur le site; 2019.

Avaliable:http://www.WIKIAGRI.FR Page consulté le 04/08/2020

- FAO. Food and agricultural organization of the United Nations; 2018. Avaliable:http://www.fao.org/faostat/fr/#dat a/QC/visualize. Site consulté le 15/12/2020
- Harold M, Tabo R. Les cultures céréalières: riz, maïs, millet, sorgho et Blé, Nourrir l'Afrique. 2015;37.
- Laopé A, Casimir S, Mariame C, Roger B, Tah VFN, Kouadio AFB. Response of two local varieties of maize (*Zea mays* L.) to two types of fertilization in post-floral water deficit conditions in sudanian zone of Côte d'Ivoire. International Journal of Innovation and Applied Studies. 2020;29(3):443-455.
- Lobell DB, Bänziger M, Magorokosho C, Vivek B. Nonlinear heat effects on African Maize as evidenced by historical yield trials, Nature Climate Change. 2011;1:42-45.
- Schlenker W, Lobell DB. Robust negative impacts of climate change on African Agriculture. Environmental Research Letters. 2010;5:1-9.
- Lichtenthaler HK. Chlorophyll and carotenoids: Pigments of photosyntetic biomembranes. Methods in Enzymology. 1987;148:350–382.
- 9. Singh. Biochemistry of phenolic compounds, (ed), Academic press, London-New York; 2000.
- 10. Bradford. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principal protein dye binding. Ann, Brochem. 1976;72:248-254.
- 11. Dreir W, Goring M. Der einfluss hoher salzkon zentratimen auf verschieden physiologische parameter von maïswuzeen, Win Z, der HU Berlin. Nath, Naurrwiss Revue. 1974;23:641-644.
- 12. Sergio AF, María FBZ, Jesús AGM, Carolina LC, Angel EPG, Fannie IPC, Sergio SV. Gamma radiosensitivity study on wheat (*Triticum turgidum* ssp. durum). Open Agriculture. 2020;5:558–562.
- Ilyas S, Naz S. Effect of gamma irradiation on morphological characteristics and isolation of curcuminoids and oleoresins of curcuma longa I. The Journal of Animal & Plant Sciences. 2014;24(5):1396-1404.
- Saiful AFMI, Muzahedul MI, Mehedi HMd. Effect of Gamma Irradiation Doses on Morphological and Biochemical Attributes of Grape Saplings. Agricultural Sciences. 2015;6:505-512.

- Rozman L. The effect of gamma radiation on seed germination of barley (*Hordeum vulgare* L). Acta Agriculturae Slovenica. 2014;103(2):307–311.
- Hafsa A, Zoya G, Sandal S, Alvina G. Effects of Gamma Radiation on Crop inadequacy gap in vulnerable populations. Advances in Nutrition. 2016;2:217–224.
- Tumuluri S, Syed HR. Effect of uv-a radiation on photosynthetic pigments of selected crop Plants, Biolife. 2014;2(1):381-386.
- He J, LU D, YU L, LI W. Pigment analysis of a color-leaf mutant in Wandering Jew (*Tradescantiafluminensis*) irradiated by carbon ions. Nuclear Science and Techniques. 2011;22:77-83.
- Abd El-Rahman AEI-RA, Gamal KA, Helmy El-SH, Hend NM. Effect of Gamma Radiation on Oil Quality of Dried Mint Leaves. American Journal of Agricultural and Biological Sciences, 2020; 15(1):83-97.
- Fereshteh EG, Abdollah RG, Taghi G. Effect of gamma irradiation on the total phenolic content and free radical scavenging activity of iranian date palm mazafati (*Phoenix dactylifera* I,). International Journal of Latest Research in Science and Technology. 2015;4(5):149-153.
- Fabiana KH, Adriana DTF, Juliana MAS, Thaise CFN, Natascha SG, Ursula MLM, Susy FS. Influence of gamma irradiation on phenolic compounds of minimally processed baby carrots. International

Nuclear Atlantic Conference, Recife, PE, Brazil. 2013;24-29.

- 22. Fatehia P, Alamouti AA, Behgar M, Norouziana AM. Effect of electron irradiation on some physical, chemical and digestion properties of pistachio byproducts. Radiation Physics and Chemistry. 2020;174:108921.
- 23. Dehpour AA, Gholampour M, Rahdary P, Talubaghi JRM, Hamdi MMS. Effect of gamma irradiation and salt stress on germination, callus, protein and proline in rice (*Oryza sativa* L,).Iranian Journal of Plant Physiology. 2011;1(4):251-256.
- Hanafy AAH, Ghalab ARM, Hussein OS, El-Hefny AM. Effect of Gamma Rays and Salinity on Growth and Chemical Composition of *Ambrosia maritima* L, Plants. Journal of Radiation Research and Applied Sciences. 2011;4(A):1139–1162.
- Ashrafijou M, Noori SAS, Darbandi IA, Saghafi S. Effect of salinity and radiation on proline accumulation in seeds of canola (*Brassica napus* L,). Plant Soil Environ. 2010;56(7):312–317.
- 26. O'neill PM, Shanahan JF, Schepers JS. Use of chlorophyll fluorescence assessments to differentiate corn hybrid response to variable water conditions. Crop Science. 2006;46:681-687.
- 27. Djerroudi ZO, Belkhodja M, Bissati S, Hadjadj S. Effet du Stress Salin sur l'accumulation de Proline chez deux espèces d'*Atriplex halimus* L, et *Atriplex canescens*, European Journal of Scientific Research. 2010;41:249-260.

© 2021 Koutoua et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/72653