

The Effect of Seed Size on Phytochemical Composition in Cowpea Lines (*Vigna unguiculata* (L.) Walp.) from Burkina Faso

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How to cite this paper: Sombié, P.A.E.D., Sama, H., Barro, A., Hilou, A. and Kiendrébéogo, M. (2021) The Effect of Seed Size on Phytochemical Composition in Cowpea Lines (*Vigna unguiculata* (L.) Walp.) from Burkina Faso. *Agricultural Sciences*, 12, 1462-1472.

<https://doi.org/10.4236/as.2021.1212093>

Received: October 23, 2021

Accepted: December 18, 2021

Published: December 21, 2021

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Abstract

The study aimed to determine the effect of seeds size (length, width, thickness and 100 seeds-weight) on their phytochemical composition (total phenolics, total flavonoids, total proteins, soluble sugars and starch) of cowpea accessions (*Vigna unguiculata* (L.) Walp.) grown in Burkina Faso. The genotypes and their descendants had length, width, thickness and 100 seeds weight between 80 ± 0.7 to 7.40 ± 0.46 cm; 6.51 ± 0.41 to 5.76 ± 0.46 cm; 5.15 ± 0.37 to 4.22 ± 0.22 cm; 12.93 ± 0.09 to 20.16 ± 0.39 g respectively. Total phenol, total flavonoid and starch contents ranged from 585.37 ± 9.73 to 1704.71 ± 4.87 mg GAE per 100 g DW; 41.42 ± 2.03 to 52.26 ± 0.75 mg QE per 100 g DW; $6.45\% \pm 0.3\%$ to $46.58\% \pm 0.12\%$ respectively. The soluble sugars and total proteins contents were ranged respectively from 149.33 ± 3.80 to 432.82 ± 40.81 $\mu\text{g}/100\text{mg}$, and 20.28 ± 0.17 to 27.32 ± 0.17 (%). Seeds size characters had significant and positive correlations with starch content, and significant and negative correlations with total phenolic content. The study of the genetic parameters of phytochemical traits also revealed that they are slightly influenced by the environment. Genetic factors are believed to account for the variation in the observed seed traits, which provides a high potential of studied accessions for breeding programs.

Keywords

Cowpea, Seeds Size, Phytochemistry, Genetic Parameters

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp., a diploid species with $2n = 2x = 22$ chromosomes is an important staple food crop in Africa because of its high protein content and its tolerance for sandy soil and low rainfall [1]. Also known as cowpea, it is a dicotyledonous grain legume that belongs to the Fabaceae family [2]. 91% of the global production was provided by Africa alone [1]. Cowpea seeds are the major products for consumption, and seed size is an important component of seed yield and also an essential market trait [3]. Seed weight is the most discriminating variable and accounts for 93% variance in physical properties [4]. Food grains have been subjected since the beginning of agriculture to selection and breeding for size and the most grains consumed today are larger than their wild relative [5]. It is already shown that seed weight influences dispersal ability and seedling establishment, and large seeds also contain enough nourishing substances for germination and have better tolerance to abiotic stresses [6] [7]. It is reported that many genes govern cowpea seed weight (size) by acting mainly additively with small size partially dominant over large [3]. TTG2 (TRANSPARENT TESTA GLABRA2), AP2 (APETALA2), ARF2 (AUXIN RESPONSE FACTOR2)/MNT (MEGAINTEGUMENTA), DA1, KLU (KLUH)/CYP78A5 (CYTOCHROME P450 78A5) and DA2 are some characterized maternal factors acting in the seed coat to influence seed size [8]. In Arabidopsis and rice, ubiquitin pathway, transcription factors, G-protein signaling, and hormone signaling are involved in maternal control of seed size [7]. Seed size variation is remarkably large among the different cowpea genotypes from Burkina Faso. The relation between seeds size and biochemical composition of cowpea is not well understood. Therefore, the aim of this study was to assess the effect of seed size on phytochemical composition in some cowpea accessions (*Vigna unguiculata* (L.) Walp.) from Burkina Faso.

2. Material and Methods

2.1. Plant Material

The seeds of five lines of cowpea were used in this study. The seeds of cowpea varieties were classified into five groups based on the seed size as very small, small, and medium, large and very large. The lines used based on seed size were classified as follows: IT81D-994: very large, Yiisyandé: large, Komcallé: medium, Niizwe: small, Melakh: very small. IT81D-994 line is the parent who transmitted the grain size character, Mélakh the parent with the very smallest seeds and the other Parents were constituted of Yiisyandé, Komcallé and Niizwe lines. Four descendant plants BC2F1 (hybrid heterozygous): [Yiisyandé × IT81D-994] Yiisyandé; [Niizwe × IT81D-994] Niizwe; [Mélakh × IT81D-994] Mélakh; [Komcallé × IT81D-994] Komcallé and four descendants plants BC2F2 (homozygous) [Yiisyandé × IT81D-994] Yiisyandé; [Niizwe × IT81D-994] Niizwe; [Mélakh × IT81D-994] Mélakh; [Komcallé × IT81D-994] Komcallé resulting from various

retro-crossings were also used in this study.

2.2. Field Experiment

2.2.1. Obtaining the Retro-Crossing Plants

To cross two varieties in cowpeas, one was chosen as the male parent and the other as the female parent. In these cases, a large number of crosses were made to obtain the maximum of grains. The crossing consisted in making an incision of about 2/3 the width of the unhatched button of the plant chosen as female (Yiisyandé, Niizwe, Mélakh, Komcallé); gently detaching the incised outer portion; ablating all the stamens of the flower of the variety chosen as female without bursting them and pollinating the emasculated flower with pollen from the flower of the variety chosen as male (IT81D-994). At maturity, only the pods from a cross were harvested. The pods were then dried, shelled and the seeds packaged in envelopes with the date, type of cross and varieties. The envelopes were then kept in the CREAM-Kamboinsé (INERA) cold room until the experiment was carried out in the Laboratory of Biochemistry and Chemistry Applied (LABIOCA), University Joseph KI-ZERBO, Burkina Faso. The male parent was chosen for the grain size. After the step of creating the F1 individuals, it was proceeded to the creation of the BC1F1, BC2F1 individuals using this time the F1 and BC1F1 individuals as female parents and the Yiisyandé, Niizwe, Mélakh, Komcallé varieties as male parents in order to keep the maximum of their characters in the descendants. BC1F1 individuals were then self-fertilized and gave birth to BC2F2 individuals who were homozygous. The crosses were made in a greenhouse of Saria (INERA) protected from insects.

2.2.2. Agro-Morphological Evaluation

Agro-morphological traits of seeds of different lines and descendants of cowpea were assessed. The studied parameters included the length, width and thickness of seed, and 100 seeds weight were determined. The measurement of length, width and thickness of five groups of twenty (20) seeds each randomly selected were done using an electronic Vernier caliper (precision of 0.01 mm) and five groups of hundred seeds weight using an electronic balance (precision 0.001 g).

2.3. Biochemical Parameters

2.3.1. Moisture and Ash Content

The moisture was determined by drying in an electric oven at 110°C until a constant weight was obtained. Moisture content was then calculated as per cent water loss. The ash content was determined after calcination in a muffle furnace at 550°C.

2.3.2. Soluble Sugars Content

The seeds of cowpea of different lines of cowpea (500 mg) were homogenized in 5 ml of hot ethanol (80%). The homogenate was centrifuged after cooling at 4000 rpm for 10 min. The supernatant was used to estimate the soluble sugar

content of cowpea seeds.

The soluble sugars content of the cowpea seed extracts was determined at 490 using the phenol-sulfuric acid method as described by Dubois *et al.* [9]. The total sugar content was expressed as μg glucose equivalent/100milligram of seeds dried weight ($\mu\text{g GE}/100\text{mg}$ of seeds DW).

2.3.3. Total Starch Content

Total starch content was determined using iodine colorimetric method as described by Jarvis *et al.* [10]. The total starch content was expressed as g/100g of seeds dried weight.

2.3.4. Protein Content

The protein concentration was determined by the Kjeldahl method as described by Philomena *et al.* [11]. The total N_2 was determined and multiplied by the factor 6.25.

2.3.5. Total Phenolics and Total Flavonoids Contents

500 mg of seeds powder of each cowpea line were extracted with 10 ml of acetone: water (80:20 v/v). The mixture was shaken for 24 h and centrifuged at 4500 rpm for 15 minutes. The supernatant was used for the quantification of total phenolics and total flavonoids contents.

The total phenolics and total flavonoids contents of seeds extracts were determined respectively at 760 nm and 415 nm using the methods described by Sombié *et al.* [12]. The total phenolics were expressed as mg of gallic acid equivalent per 100 gram of seeds dried weight (mg GAE/100g of seeds dried weight) and total flavonoids expressed as mg of quercetin equivalents (QE) per 100 gram of seeds dried weight (mg QE/100g of seeds dried weight).

2.4. Statistical Analysis

The results are presented as mean \pm SD for triplicate analysis. Two-way analysis of ANOVA variation followed by Tukey's Significant Difference test was used to compare the results and $P < 0.05$ was considered significant. The Pearson correlation test was used to study the different correlations between the agro- morphological and biochemical parameters.

Genetic parameters were calculated using formula proposed by Falconer and Mackay [13]. The measured variables were processed by analysis of variance to determine the genotype effect. The measured population variables are processed by descriptive statistical analysis, to obtain the means, minimum and maximum extreme values, amplitude and phenotypic variances of the populations. Components of variation are deduced to estimate coefficients of phenotypic (CVP) and genotypic (CVG) variability and the degree of heritability of traits measured by the ratio of phenotypic and genotypic variances to the trait mean:

$$\text{CVP}(\%) = \frac{100 * \sigma^2}{X}; \quad \text{CVG} = \frac{100 * \sigma^2}{X}$$

CVP: coefficients of phenotypic variation; CVG: coefficient of genotypic variation; σ^2 is either the total phenotypic or genotypic variance deduced from the analysis of variance by equating the mean expectations with the mean squares of the variances; \bar{X} is the overall mean of the trait.

These same components are estimated, for the variables measured by population, on the basis of the calculation of the variances of the F2, taken as equal to the total phenotypic variance ($\sigma^2 p$), the variance of the parents is taken as equal to the residual variance ($\sigma^2 e$), and the genetic component of this variance which is deduced by difference:

$$\sigma^2 G = \sigma^2 p - \sigma^2 e$$

Heritability in the broad sense ($h^2 BS$) is estimated by the ratio of genetic variance to total phenotypic variance: $h^2 BS = \sigma^2 G / \sigma^2$.

3. Results and Discussion

Table 1 summarizes the effect of varietal difference on agro-morphological traits of cowpea seeds. There were statistical differences in seed length, width, thickness and 100 seeds weight among the genotypes at the $p < 0.05$ level.

The length (cm) of cowpea seeds lines ranged from 7.40 ± 0.46 to 9.80 ± 0.7 cm. Seeds from the parent IT 81D-994 showed the significantly higher seed length values than the rest of the genotypes. The lowest value of length found was in the parent Melahk genotype, but it was not significant relative to the descendant BC2F2 (Melahk \times [IT 81D-994] Melahk) seeds (7.45 ± 0.38 cm).

Cowpea IT 81D-994 (6.51 ± 0.41 cm), Yiisyandé (6.48 ± 0.34 cm), Niizwé (6.46 ± 0.35 cm) and Komcallé (6.46 ± 0.35 cm) lines recorded higher mean values for width (cm). The parent melahk (6.03 ± 0.43 cm) genotype showed the significant same value for width with all the BC2F2 descendants. The seeds of Yiisyandé genotype (5.15 ± 0.37 cm) showed no significantly higher mean value for thickness when compared to those of IT 81D-994 (5.11 ± 0.45 cm), Niizwé (4.87 ± 0.33 cm) and Komcallé (4.85 ± 0.35 cm) genotypes. The descendant

Table 1. Agro-morphological trait of cowpea seeds.

Type of Cross	Cowpea lines	Length (cm)	Width (cm)	Thickness (cm)	100 seeds weight (g)
Parent	IT 81D-994	$9.80 \pm 0.7a$	$6.51 \pm 0.41 a$	$5.11 \pm 0.45 a$	$20.16 \pm 0.39 a$
	Yiisyandé	$9.18 \pm 0.74b$	$6.48 \pm 0.34 a$	$5.15 \pm 0.37 a$	$19.24 \pm 0.23 b$
	Niizwé	$8.72 \pm 0.42 bc$	$6.46 \pm 0.35 a$	$4.87 \pm 0.33ab$	$14.85 \pm 0.25 d$
	Komcallé	$8.88 \pm 0.76 bc$	$6.46 \pm 0.35 a$	$4.85 \pm 0.35 ab$	$18.77 \pm 0.25 b$
	Melahk	$7.40 \pm 0.46 d$	$6.03 \pm 0.43 b$	$4.46 \pm 0.32 cd$	$13.76 \pm 0.34 e$
BC2F2	Yiisyandé \times [IT 81D-994] Yiisyandé	$8.92 \pm 0.81 bc$	$5.76 \pm 0.46 b$	$4.69 \pm 0.47 bc$	$17.74 \pm 0.06 c$
	Niizwé \times [IT 81D-994] Niizwé	$8.33 \pm 0.52 c$	$5.84 \pm 0.26 b$	$4.38 \pm 0.29 cd$	$15.18 \pm 0.21 d$
	Komcallé \times [IT 81D-994] Komcallé	$8.63 \pm 0.55 bc$	$6.04 \pm 0.30 b$	$4.59 \pm 0.33 bc$	$17.10 \pm 0.24 c$
	Melahk \times [IT 81D-994] Melahk	$7.45 \pm 0.38 d$	$5.87 \pm 0.32 b$	$4.22 \pm 0.22 d$	$12.93 \pm 0.09 f$

BC2F2 (Melahk × [IT 81D-994] Melahk line recorded the weak thickness (4.22 ± 0.22 cm) but not significantly different with those of the parent Melahk (4.46 ± 0.32 cm) and the descendant BC2F2 (Niizwé × [IT 81D-994] Niizwé (4.38 ± 0.29 cm).

The 100 seeds weight (g) of the different lines varied from 12.93 ± 0.09 to 20.16 ± 0.39 g. The IT 81D-994 genotype showed the highest 100 seeds weight and the descendant (Melahk × [IT 81D-994] Melahk) showed the weakest 100 seeds weight.

According to Davies and Zibokere [4] cowpea varieties' seed size was divided into four categories based on their 100 seeds weight: Varieties with seeds 10 - 15 g are described as small, 15.1 - 20 g as medium-sized seeds while large seeds have 20.1 - 25.0 g. Seeds weighing over 25 g are described as very large seeds.

Based on this classification, the parent IT 81D-994 will be classified as variety with large seeds. The parents Yiisyandé and Komcallé, and the descendants BC2F2 (Yiisyandé × [IT 81D-994] Yiisyandé, BC2F2 Niizwé × [IT 81D-994] Niizwé and BC2F2 Komcallé × [IT 81D-994] Komcallé will be classified as genotypes with medium size seeds. Niizwé, melahk and the descendant BC2F2 Melahk × [IT 81D-994] Melahk are genotypes with small seeds. Any variety with very large seeds was not found in this study.

The variation observed for seeds size in this study might be attributed to crop growth, the development and maturation of seed, and of the inherent genetic differences among different lines [14].

When **Table 2** is examined, statistically significant differences ($p < 0.05$) should be seen between the lines of cowpea in terms of total phenolics, total flavonoids, starch, soluble sugars and total proteins contents.

The seeds of the BC2F2 [Komcallé × IT81D-994] Komcallé line contained significantly the higher level of total phenolics than all the other lines of *V. unguiculata* reaching 1704.71 ± 4.87 mg GAE per 100 g DW. Komcallé line had the

Table 2. Phytochemical compounds content of cowpea lines.

Type of cross	Cowpea lines	Total Phenolic (mg/100g)	Total Flavonoids (mg/100g)	Starch (%)	Soluble Sugars (µg/100mg)	Total Proteins (%)
Parent	IT 81D-994	873.17 ± 4.87 e	44.09 ± 1.73 bc	23.53 ± 0.09 c	246.72 ± 23.52 c	23.71 ± 0.42 b
	Yiisyandé	597.05 ± 19.38 g	52.26 ± 0.75 a	24.71 ± 0.07 b	168.65 ± 8.67 ef	23.97 ± 0.17 b
	Niizwé	683.07 ± 6.37 f	44.76 ± 0.85 bc	17.29 ± 0.36 e	174.74 ± 3.60 ef	27.32 ± 0.17 a
	Komcallé	585.37 ± 9.73 g	44.41 ± 1.63 bc	46.58 ± 0.12 a	338.11 ± 10.27 b	21.45 ± 1.68 cd
	Melahk	992.11 ± 14.60 d	46.16 ± 3.81 abc	19.27 ± 0.73 d	149.33 ± 3.80 f	20.28 ± 0.17 d
BC2F2	[Yiisyandé × IT81D-994] Yiisyandé	1235.31 ± 7.36 c	49.14 ± 3.46 ab	19.34 ± 0.12 d	227.35 ± 13.12 cd	23.80 ± 0.33 b
	[Niizwé × IT81D-994] Niizwé	610.86 ± 9.73 g	41.42 ± 2.03 c	12.07 ± 0.55 g	432.82 ± 40.81 a	23.80 ± 0.33 b
	[Komcallé × IT81D-994] Komcallé	1704.71 ± 4.87 a	47.34 ± 1.82 abc	15.75 ± 0.18 f	190.41 ± 7.69 def	22.71 ± 0.08 bc
	[Melahk × IT81D-994] Melahk	1271.42 ± 20.48 b	49.95 ± 0.16 ab	6.45 ± 0.3 h	206.21 ± 4.98 cde	25.98 ± 0.17 a

lowest total phenolic content of 585.37 ± 9.73 mg GAE per 100 g DW. The highest total flavonoids content was obtained in the Yiisyandé line (52.26 ± 0.75 mg QE/100g) and the lowest content was found in the BC2F2 [Niizwé \times IT81D-994] Niizwé line (41.26 ± 2.03 mg QE/100g).

The cowpea lines exhibited a wide range of variation in seed starch (%). The majority of parent genotypes exhibited high starch content. The average content of seeds starch varied from $46.58\% \pm 0.12\%$ in Komcallé to $6.45\% \pm 0.3\%$ in [Melahk \times IT81D-994] Melahk. Results revealed a higher concentration of soluble sugars in [Niizwé \times IT81D-994] Niizwé, while Melahk exhibited the lowest amount of soluble sugars accumulation respectively of 432.82 ± 40.81 and 149.33 ± 3.80 $\mu\text{g}/100\text{mg}$. Significant differentiation was observed in total proteins concentration within the studied cowpea lines. The cowpea lines had a wide range of variation in total proteins ranging from 27.32 ± 0.17 to 20.28 ± 0.17 (%). The varieties with the greatest total proteins were Niizwé ($27.32\% \pm 0.17\%$) and [Melahk \times IT 81D-994] Melahk ($25.98\% \pm 0.17\%$) and those with the lowest was Melahk ($20.28\% \pm 0.17\%$).

3.1. Genetic Parameters

Table 3 summarizes the genetic parameters of the phytochemical parameters of the seeds of the different varieties. The results showed variations in the phenotypic and genotypic coefficients of variation according to the studied parameters. In general, and for all parameters studied, the phenotypic coefficients of variation were slightly higher compared to the genotypic ones. Furthermore, only water and starch content showed low genotypic and phenotypic coefficients ($\leq 10\%$). The other parameters showed high coefficients ($\geq 20\%$). The existence of significant differences among the genotypes for all the seeds studied traits, indicates the presence of considerable genetic variability among the different varieties. This variability in seeds traits could offer many opportunities to improve different quantitative and qualitative traits through breeding [15] [16].

Table 3. Genetic parameters of the phytochemical compounds of the seeds.

Traits	σ^2g	σ^2p	σ^2e	Mean	GCV (%)	PCV (%)	Hbs	GA (%)	GAM
Water content	0.053	0.052	0.001	11.362	0.005	0.068	0.994	0.228	2.007
Ash	0.031	0.031	0.000	3.369	0.096	0.310	0.989	0.174	5.169
Total flavonoids	11.473	9.960	1.513	46.614	0.496	0.704	0.868	2.940	6.308
Total phenolics	150.721	49.480	101.241	950.343	0.398	0.631	0.328	4.030	0.424
Starch	12.973	10.299	2.674	20.555	0.794	0.891	0.794	2.860	13.911
Total sugar	85.135	75.199	9.936	237.149	0.599	0.774	0.883	8.150	3.437
Proteins	4.510	4.387	0.123	23.667	0.437	0.661	0.973	2.066	8.729
100 seeds weight	6.561	6.540	0.021	16.636	0.628	0.792	0.997	2.553	15.349

σ^2g = Genotypic variance, σ^2p = Phenotypic variance and σ^2e = Environmental variance, Hbs = Heritability broad sense, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, GA = Genetic advance, GAM = Genetic advance as percent of mean.

The higher phenotypic than genotypic coefficients of variation would indicate that the phenotypic variation of the observed traits was less influenced by the environment. In addition, the high values for the high phenotypic and genotypic coefficients of variation (20%) suggest that selection on the basis of the relevant traits would be effective, as there is a good correlation between measured phenotypic and genotypic expression [17]. Indeed, for Admas and Tesfaye [16] the coefficients of phenotypic (CVP) and genotypic (CVG) variability are classified as high (above 20%), medium (between 10% and 20%) and low (below 10%). In the case of our study, apart from moisture content and total ash, which have low genotypic coefficients, the other traits (coefficients above 20%) would have good potential for varietal improvement programmes. Similar results were reported by Bartaula *et al.* and, Admas and Tesfaye [15] [17].

All the studied parameters excluding the phenolic compounds showed high values of heritability in the broad sense ($\geq 60\%$). Genetic advance as a percent of mean showed values ranging from 2% to 15.349%. Thus, genetic advances as a percent of mean are low for water, total ash, total flavonoids, total polyphenols, total sugars and total proteins which showed values below 10%. Only starch and 100-seed weight showed high genetic advance. Our studied traits in general show high broad heritability coupled with low genetic advance as a percent mean. Such results suggest expression of the trait is under the control of non-additive type of gene action, and its response to selection would be poor. Indeed, according to Bartaula *et al.* [15], traits which expressed high heritability coupled with high genetic advance indicate the preponderance of additive gene action in controlling the traits. Hence direct selection of such characters would be effective in improving the yield. However, traits with high heritability coupled with low genetic advance, suggest expression of the trait is under the control of non-additive type of gene action, and its response to selection would be poor. Those with lower heritability as well as genetic advance suggesting the trait governance by non-additive gene action and direct selection would not be effective for the trait [15].

3.2. Phytochemical Compounds Contribution to the Seeds Size in Cowpea Lines (*Vigna unguiculata* (L.) Walp.) from Burkina Faso

Pearson correlation matrix between the phytochemical and seeds size in cowpea lines was performed and presented in **Table 4**. Significant relationship between starch content and seeds size (weight, length, width and thickness) were observed. There were strong positive and significant correlations between the starch content and the weight ($r = 0.639$, $p < 0.05$), the starch content and the length ($r = 0.467$, $p < 0.05$), the starch content and the width ($r = 0.611$, $p < 0.05$), the starch content and the thickness ($r = 0.585$, $p < 0.05$). Furthermore, negative correlations were also found between the total phenolic content and seeds size. In contrast, weak correlations were observed between the total flavonoids and

Table 4. Pearson correlation coefficient.

	Total flavonoids	Total phenolics	Starch	Sugars	Proteins
100 seeds Weight	0.057	-0.206	0.639	0.123	-0.220
Length	-0.081	-0.267	0.467	0.134	0.106
Width	-0.074	-0.540	0.611	-0.171	0.067
Thickness	0.105	-0.434	0.585	-0.183	0.041

seeds size, between sugars content and seeds size and between proteins content and seeds size. Size and chemical composition are important aspects of the quality of seed [18].

This study showed that the seed weight of cowpea variety could be a useful criterion for determining suitability for a particular end-use application. Varieties with large seeds would be preferred for high starch content, however seeds with small width would be preferred for high total phenolic content.

4. Conclusion

In this study, the cowpea accessions exhibited variations of phytochemical constituents according to the size of their seeds. The larger size of seeds from the cowpea accession was accompanied by higher content of starch; however the seeds with small size achieved the highest total phenolic content. The study showed that the size of the seeds has no influence on their soluble sugars, total proteins and total flavonoids contents. It is suggested that the results reflect the influence of inheritance on the seeds size and phytochemical composition of cowpea.

Acknowledgements

The research work has been done with the financial support of International Centre for Genetic Engineering and Biotechnology (CRP-ICGEB BFA 20-01) and The World Academy of Sciences (TWAS NO 20-155 RG/BIO/AF/AC_I-FR 3240314143) individual Fellowship Grant granted to the first author

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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