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Segregation of Genes Controlling Seed Coat Color in Cowpea (*Vigna Unguiculata* [L] Walp)

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Abstract

Cowpea coat color is a consumer-related trait that determines consumer preference and acceptability of the commodity in the market. Understanding the mode of inheritance of seed coat color will assist in choosing appropriate breeding technique in developing desirable cowpea varieties. This study was conducted to confirm the specific mode of inheritance of seed coat color in cowpea. Three bi-parental crosses using five different varieties of cowpea with contrasting seed coat colors were carried out to obtain F_1 seeds at the Greenhouse, Faculty of Agriculture, Obafemi Awolowo University, Ile – Ife, Nigeria. The F_1s were advanced to obtain F_2 segregating populations which were evaluated by visual observation and the seeds were categorized into different distinct groups. The observed seeds of the F_2 population of all the three crosses deviated extremely from Mendelian monogenic inheritance ratio in different degrees. TVu4669 × TVu43 segregated into two color groups resulting in brown mottled (15.4 %) and black mottled (84.6 %). Ife Brown × TVnu699 segregated transgressively into five distinct groups of colors giving rise to brown (51.4%), black (23.7%), grey (14.8%), red (5.3%) and speckled (4.8%); while Ife Brown × TVu2723 segregated into three color groups involving mottled black (4.3%), black (85.1%) and speckle (10.6%) coat colors. It could be inferred that more than one gene controls the inheritance of the seed coat color in cowpea, which implies that the trait is polygenic. It is recommended that molecular genetics' techniques be explored in order to have better understanding of the mode of inheritance of seed coat color in cowpea.

Key Words: Coat color, cowpea, qualitative trait, quantitative trait, monogenic inheritance ratio.

Introduction

Cowpea [*Vigna unguiculata* (L) Walp] (2n = 22) is believed to be native to Africa (Tan *et al*., 2012; Jayathilake *et al*., 2018). This was based on the fact that wild species of cowpea are found throughout African countries**.** Cowpea is a major food crop for many people around the world as well as reared animals especially in developing countries such as most African countries (Fatokun *et al*., 2002) where the largest proportion of the world's production, trade and consumption take place. The crop is known for its high nutritional content of about 23–25% protein, 6.3% fiber and 50–67% starch (Yeung *et al*., 2009; Tejashree and Dalvi, 2015; Stancheva *et al*., 2016). The protein content could even be as high as 36.75% in the grains of some varieties (Olotuah and Fadare, 2012). Nutritional value of cowpea is of course not just limited to dry grain contents mentioned above; but it also contains considerable amount of Sodium, calcium, magnesium and potassium and well as iron and zinc which are important macronutrients and micronutrients needed by human being although, the amount present vary from variety to variety (Asante *et al*., 2006; Santos and Boiteux, 2013). It is on this note scientists need to put in more efforts not only to make cowpea available for the populace, but to developing varieties that are also appealing to the consumers but in visual attraction and content robustness.

Generally, the primary goal of crop improvement is usually to target yield and its components cum quality traits that determine the crop acceptability to both farmers and consumers. Yield is ultimate in plant breeding (Obisesan, 2003); and it is on this feat that the introgression of other traits such resistance to both abiotic and biotic stresses, nutrient and other quality traits can be achieved via appropriate breeding methods. Nearly all cowpea improvement programs focus on higher grain yields and improved quality as primary breeding objectives (Timko and Singh, 2008). It is important to note that developing a new superior variety (high yielding and big seed size) that lack consumers' acceptability is equivalent to a waste of time, resources and efforts for breeders. On this wise, scientists' efforts are being strategically put in place to make research count by adopting participatory crop improvement methods where all inputs of all stakeholders – including farmers and consumers are put into consideration.

Seed coat color is one of the major physical characteristics that determine the preference of any consumer hence, it is regarded as an important consumer-related trait in cowpea (Nwofia, 2014; Herniter *et al*., 2018). Previous research has indicated that consumers make decisions on the acceptability, quality, and presumed taste of a product depending on appearance, especially color (Simonne *et al*., 2001). Different colors have been reported in cowpea such as white, brown, blue, red, maroon, green, black, even mottled and holstein type cowpea such as white and black color in TVu2723 line (Singh and Ishiyaku, 2000; Reda *et al*., 2016). Research has shown that consumer preferences vary from one region to another. A rough seed coat is ideal in west and central African countries because it permits easy removal of the seed coat which is important for indigenous food recipes. In contrast, a smooth seed coat is preferred in eastern and southern Africa and also in parts of South America where cowpea is consumed as cooked beans without removing the seed coats.

Studies on inheritance have been conducted to decode mode of inheritance on some important traits on cowpea. Egbadzor (2014) used different bi-parental crosses to estimate the number of genes that might control seed coat color in cowpea in order to decipher its mode of inheritance. He concluded that many genes were responsible for controlling coat color, arguing that many anthocyanins are known to be present in the seed coat of cowpea, and anthocyanin content of the various parts of a plant can be quantified. This implies that the pigmentation of the seed coat of cowpea can also be quantified, this result was in line with the report published by some of scientists (Mustapha, 2009; Kehinde, 2000). Arguments are still ongoing among researchers to ascertain whether cowpea coat color is qualitatively or quantitatively inherited. Furthermore, of all the genetic research on cowpea seed coat color that has been carried out, very few has used speckled and/or mottled cowpea variety. There is therefore need to categorize the seed coat color in cowpea to either qualitative or quantitative in a bid to decipher its mode of inheritance. The objective of this study was to confirm or refute whether seed coat color in cowpea is truly monogenic.

Materials and Methods

The genetic materials used in this study included five cowpea genotypes obtained from the International Institute of Tropical Agriculture, IITA Ibadan and Department of Crop Production and Protection in Obafemi Awolowo University, Ile – Ife. The names and characteristics are presented in the Table 1.

Table 1. List of cowpea varieties used in this study, their colors, flowering traits and sources

S/N	Varieties	Seed coat color	Days to flowering	Source
1	*TVu4669	Mottled white	46	IITA
2	*TVu2723	Holstein	40	IITA
3	*TVu 43	Black	43	IITA
4	**TVNu699	Grey	44	IITA
5	Ife Brown	Brown	41	Ile – Ife

*TVu - Tropical *Vigna unguiculata*, *TVNu -Tropical None *Vigna unguiculata*

Bi-parental crosses

Three crosses were carried out from the five true breeding cowpea genotypes. The crosses included: TVu 4669 x TVu 43, Ife Brown x TVNu699 and Ife Brown x TVu2723. Plastic pots were filled with top soil; four seeds of each genotype were planted per pot and three pots per variety in the greenhouse. After two weeks, another set of pot, one for each variety was planted to facilitate synchronous flowering since flowering date for each variety differ. Two weeks after planting, seedlings were thinned to two stands per pot. Staking was done to support the plants. Cypermethrin was used to control aphids (*Aphis craccivora*) and other insect pests in the greenhouse and in the field, NPK 15:15:15 fertilizer was used in the greenhouse to supplement the soil nutrient, regular hand weeding and as well as watering at every other day to ensuring the plants were well irrigated as at when due.

Cowpea is a self-pollinating species, mode of pollination is naturally selfed and therefore, cross pollination was enforced. This is done by first emasculating the mature flower buds intended to open the following day with the use of sterilized forceps. The successful crosses were managed to maturity, harvested as F_1 seeds and certain portion these seeds were allowed to self to obtain F_2 seeds. The evaluation was conducted on the field laid out in randomized complete block design with three replicates for evaluation of parents (P_1 and P_2) and F_1 population while four replicates for the F_2 population and all at spacing of 60cm x 30cm. The seeds were harvested at maturity, dried, threshed and classified seed coat color into distinct color categories using visual observation and counted separately. The plan was to group F_2 seeds according to seed coat color and test for various genetic ratios using chi-square goodness of fit to the proposed segregation ratio using SPSS. However, it was made difficult in grouping the seeds without ambiguity in many cases.

$$\chi 2 = \left[\sum (O - E)^2 / E\right]$$

Where χ^2= chi-square, O= observed values, E= expected values.

Results and Discussion

Cross 1: TVu 4669 and TVu 43

In the cross between TVu 4669 (mottled white) and TVu 43 (black), all the F_1 progenies had mottled white color of TVu 4669; while the F_1 progenies of reciprocal the same cross were hundred percent black coat color. The same observations were also noticed for all other crosses made (where the reciprocal crosses were successful). Surprisingly, none of the F_2 progenies resembled any of the parents and thus, none of the seeds could be separated into any of the two parental types in terms of coat color, hence they were categorized into two groups different from any of the parents (Plate 1). The first category was mottled brown color seeds which was 15% and the second category comprises of black mottled seeds which was 84.6 % of the total seed produced.

Table 2: Color characteristics of cowpea generations - parental lines, first and second filial generations of the cross (TVu 4669 x TVu 43).

Generation	White mottled	Black	Brown Mottled	Black mottled	Total	Expected ratio
TVu 4669	30	0	0	0	30	1
TVu 43	0	30	0	0	30	1
F_1 (TVu 4669 x TVu 43)	24	0	0	0	24	
F_2	0	0	8	44	52	
F_2 %			15.4	84.6		

Cross 2: Cross between Ife Brown (brown) and TVNu699 (grey)

All F_1 progenies had the brown coat color which was the expressed phenotype of Ife Brown- the maternal parental line. None of the twenty reciprocal crosses was successful. The F_2 (%) population exhibited transgressive segregation and an attempt to group the progenies based on color identified five groups with their respective percentages of expressions. The groups were brown (51.4%), grey (14.8 %), speckled (4.8%) red (5.3%) and black (5.3%) as presented (Table 3) while pictorial expression of the results of this cross is presented in (Plate 2). The pattern of segregation showed dominance of brown seed coat color as expressed in the results.

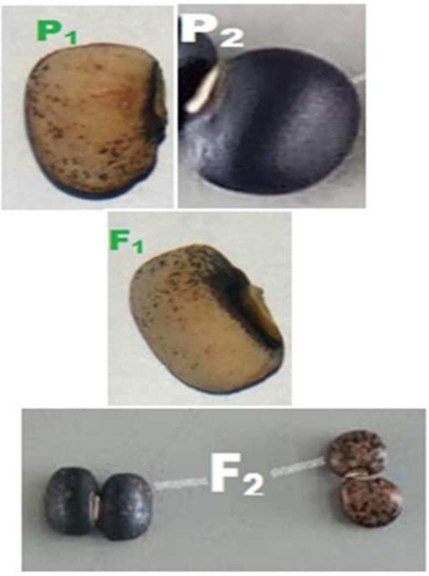

Figure 1. Colors of cowpea generations - parental lines and crosses between TVu 4669 (White mottled) and TVu 43 (black).

Table 3: Color characteristics of cowpea generations - parental lines, first and second filial generations of the cross Ife Brown (Brown) x TVNu699 (Grey)

Generation	Brown	Grey	Speckled		Black	Red	Total	Expected Ratio
Ife Brown	12	0	0		0	0	12	1
TVnu699	0	12	0		0	0	12	1
F_1	16	0	0		0	0	16	
F_2	173	50	16		80	18	337	
F_2 (%):	51.4	14.8	4.8	23.7	5.3			

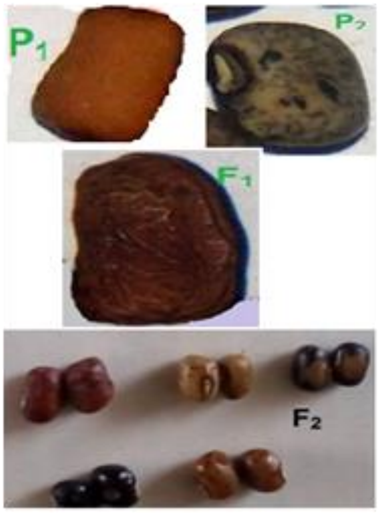

Figure 2. Colors of cowpea generations - parental lines and crosses between Ife Brown and TVNu699

Cross 3: Ife Brown (brown) and TVu2723 (Holstein-patches of white and black)

Genetic analysis of segregating populations of cowpea and tests for goodness of fit to expected ratios of cross between Ife Brown (brown) and TVu2723 (Holstein-patches of white and black) is presented in Table 4. All F_1 progenies resemble Ife Brown - the maternal parental line (i.e. were homogenous phenotypes of heterogeneous genotypes) while the F_2 seeds resembled none of the parents. The seeds segregated into three different groups of colors: black (85.1%), mottled back (4.3%) and speckled (10.6%). The pictorial representation of the phenotypes of the parental lines, F_1 and the segregation patterns of the F_2 generations are presented in Plate 3.

Discussion

There is complexity in the inheritance of seed coat color in cowpea unlike seed coat texture which follow Mendelian monogenic inheritance (Oladejo *et al*, 2020). The complexity involved in the inheritance explained the reason the test for goodness of fit among F_2 progenies in the crosses (TVu 4669 × TVu43; Ife Brown × TVnu699; and Ife Brown × Tvu2723) could not be carried out. Hence, none of the F_2 progenies resembled either parent showing intricacy in segregating pattern of seed coat color in cowpea for the two crosses - TVu4669 × TVu43 and Ife Brown × Tvu2723. Different expressions of phenotypes ranging from resemblance of maternal to paternal parents and superior performances (and non-resemblance) to the phenotypes of the two parents implied the presence of transgressive segregation in the cross - Ife Brown × TVnu699. This invariably indicated the deviation from Mendelian monogenic inheritance ratio, which suggested that more than single gene was involved in the inheritance of the trait. This invariably indicated the deviation from Mendelian monogenic inheritance ratio, which suggested that more than single gene was involved in the inheritance of the trait. Moreover, the result of the analysis of the F_2 progenies in the cross - TVu4669 × TVu43 deduced that black color is dominant to the white mottled; the expression of brown color in this cross implies that gene controlling brown is inherent in the black or white mottled genotype but was masked. The results reported by Nwofia (2014) corroborate the current findings that black and brown are dominant over white, and black is dominant over brown, these could have been the reason white color of TVu4669 was completely masked. Moreso, in the cross Ife Brown × TVnu699, the inheritance factor in the seed coat color in cowpea involved higher combinations (i.e. more pairs of independent assortment of autosomal factors) of gene pairs are responsible.

Table 4: Color characteristics of cowpea generations - parental lines, first and second filial generations of the cross Ife Brown (Brown) x TVu2723

Generation	Brown	Holstein	Mottled black	Black	Speckle	Total	Expected ratio
Ife Brown	12	0	0	0	0	12	1
TVu2723	0	12	0	0	0	12	
F_1	16	0	0	0	0	16	
F_2	0	0	6	120	15	141	
F_2 %			4.3	85.1	10.6		

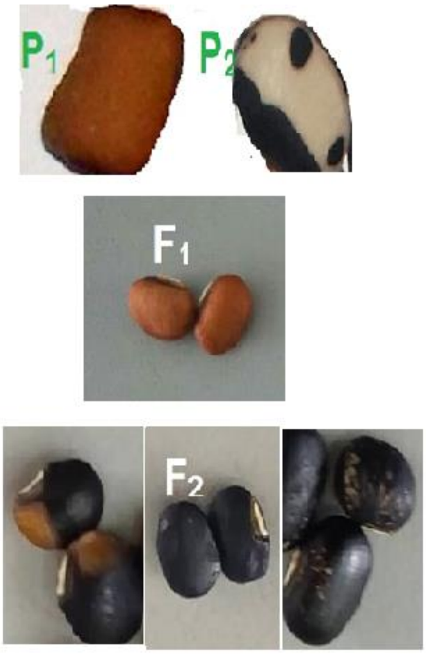

Figure 3. Colors of cowpea generations - parental lines and crosses between Ife Brown and TVNu699 Cross between Ife Brown and TVu2723

Furthermore, it was also observed that the white color in TVu2723 (holstein) in the crosses between Ife Brown and TVu2723 was masked completely and thus, establishing the fact that black and brown color are dominant over white, and this was also corroborated with previous findings (Nwofia, 2014). However, the segregation observed was totally different from what was expected, in that no single F_2 progeny of the cross resemble either parent, and neither did the observed phenotypes conform to the classical monogenic inheritance ratio. This finding is contrary to the results of the study on another seed coat character in cowpea, Oladejo *et al*., (2020) reported 'that seed coat texture is controlled by a pair of single genes and under a monogenic inheritance. They further stated that smooth seed coat texture was also observed to be dominant over rough seed coat texture in all the crosses'. In this present study, non- conformity to classical monogenic inheritance ratio could imply the effect of epistatic gene interaction or it could be as a result of genotype by environment interaction during segregation which is major feature of quantitative traits as buttressed by some scientists in earlier studies (Egbadzor *et al*. 2014); and like most morphological and phenological traits in cowpea which are multigenic, quantitative or continuous characters and their expressions are mainly influenced by environmental conditions (Oladejo *et al*., 2019). Conversely, it is noted that not all observed variations of individuals are driven by the environmental factors, as propounded in the age-long Johannsen pureline theory (Johannsen, 1909; Roll-Hansen, 2014). In this experiment, the cowpea plants were cultivated in the same environment; it could be inferred there is no significant effect of the environment on genotype (phenotype color of seeds). Invariably, this implied that the observed variations are purely genetic and the variation due to the environment factors was zero.

All these observations invariably suggested that more than a single gene might be involved in the inheritance of seed coat color in cowpea. Meanwhile, it is worthy to note that all the F_1 seeds of the three crosses expressed the color and phenotypes of the maternal parents and even with their reciprocals. Continuous variation observed in the coat color of cowpea in this study was also observed by Egbadzor *et al*. (2014) in which they crossed brown seeded UCR779 and CB27 white seeded and noticed that at F_2, ten seed coat color groups were identified, classification of these seeds according to coat color groups was arbitrary, they opined that study of seed coat color as quantitative trait might be appropriate. In another earlier study, Drabo *et al*. (1988) reported similar findings where F_2 progenies outcome could not be tested for goodness of fit. It is also noteworthy that the independent gene action for smooth seed coat and the dominance for black and brown seed colors have important implications in selecting parents for breeding if a desired cowpea variety must have brown or white seeds with rough seed coat, which is an essential requirement in western and central Africa. In that case it would be desirable to select parents for crosses having the same genes for rough seed coat and seed coat color. Otherwise either a large F_2 population would have to be screened or one or two backcrosses need to be made toward the desirable parents before evaluating the segregating populations.

Conclusion

The genetics of seed coat color of cowpea has been examined from the set of three crosses in this study. It was inferred that more than one gene controlled seed coat color of cowpea however, the exact number of genes involved in inheritance of cowpea coat color could not be determined in this study due to complexity involved in the segregation patterns. None of the crosses conformed to classical Mendelian monogenic inheritance ratio, this suggests that more than one gene is involved in the inheritance of cowpea seed coat color, which implies the trait is polygenic. In this study, color rating is basically via visual observation, it is therefore recommended that further studies on this seed trait should be conducted using sophisticated equipment *vis a vis* colorimeter which is a standard scientific instrument to measure color especially for subsequent study. Lastly, advanced studies of gene action controlling seed coat color using standard mating designs such as diallel, Generation Means Analysis and North Carolina Design 11 is recommended subsequently in order to have better understanding of the mode of inheritance of seed coat color in cowpea.

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Authors’ Contributions

This study was a collaborative work among all authors. ASO conceptualized the idea, designed the experiment and sourced for genetic materials. AOB was instrumental to the standardization of the methodology and logistics of the experiment. EOO generated the crosses in the greenhouse, genetic population, collected data, statistical and genetic analyses under supervision ASO and AOB. ASO and EOO wrote the original draft of the manuscript with invaluable inputs, corrections and guidance from RMG. ASO, AOB and RMG edited and validated the final draft of the manuscript.

Ethics

There is no any ethical issue that is associated with the conduct this study in its entirety.

References

Asante I. K. Adu-Dapaah H. and Addison P. 2006. Variation in contents of crude protein and mineral elements in 32 cowpea (*Vigna unguiculata* (L.) Walp) accessions in Ghana. *Ghana Journal of agric. Sci*. 39, 83-86.

Jayathilake C. Rizliya V., Afka D. Ruksheela B. Barana C. J. Srinivas N. and Ruvini L. 2018. Cowpea: an overview on its nutritional facts and health benefits. *Journal of Sci Food Agric*.

Drabo I. T.O. Ladeinde and Redden R. 1988. Inheritance of eye pattern and seed coat color in cowpea (*Vigna unguiculata* (L.) Walp). *Plant Breeding*, 100: 119-123.

Egbadzor K.F. 2014. Genetic control and breeding for seed size and color in wowpea [*Vigna unguiculata* (L.) Walp]. *Ph.D thesis*, Plant Breeding University of Ghana, Legon.

Egbadzor K.F. Yeboah M. Gamedoagbao D.K. Offei S.K. Danquah E.Y. and Ofori K. 2014. Inheritance of Seed Coat Color in Cowpea (*Vigna unguiculata* (L.) Walp). *International Journal of Plant Breeding and Genetics*, 8: 35-43.

Fatokun C. A. Tarawali S. A., Singh B. B., Kormawa P. M. and Tamò M. (eds). 2002. Challenges and opportunities for enhancing sustainable cowpea production. *Proceedings*

Herniter I.A. María Muñoz-Amatriaín Sassoum Lo Yi-Ning Guo. and Timothy J. Close. 2018. doi: https://doi.org/10.1534/g3.118.200521.

Johannsen W. 1909. Elemente der exakten Erblichkeitslehre. [Elements of the Exact Theory of Inheritance] Jena, Germany: Gustav Fischer.

Kehinde O.B. 2000. Segregation of genes controlling seed coat color in cowpea (*Vigna unguiculata* (L.) Walp). *Nigerian Journal of Genetics*. 15: 29-34.

Mustapha Y. 2009. Inheritance of seed coat color pattern in cowpea [*Vigna unguiculata* (L.) Walp]. *Bayero Journal of Pure Applied Science*, 2: 70-74.

Nwofia G. E. 2014. Inheritance of leaf shape, pod shape, pod color and seed coat color in cowpea (*Vigna unguiculata* L. Walp), *World Journal of Agricultural Sciences* 10 (4): 178-184.

Oladejo A.S. Bolaji A.O. and Olawuyi O.A. 2020. Inheritance pattern of seed coat texture in cowpea (*Vigna unguiculata* L. Walp). *Ife Journal of Agriculture* 32 (3): 46 -51.

Oladejo A.S. Bolaji A.O. Obisesan I.O. and Omitogun O.G. 2019. SDS-Page characterization of some elite cowpea (*Vigna unguiculata* L. Walp) varieties. *Nigerian Journal of Biotechnology. 36(2) 45 – 51.*

Olotuah O. F. and Fadare Z. O. 2012. Growth morphology and seed quality variation among five indigenous varieties of cowpea, *Vigna unguiculata*. *International Journal of Agricultural Science*. 2 (6): 546-549.

Reda M.G. Marwa H. and Abdelfattah B. 2016. Seed coat color, weight and eye pattern inheritance in gamma-rays induced cowpea M2-mutant line. *Journal of Genetic Engineering and Biotechnology* 14: 61–68.

Roll-Hansen N. 2014. Commentary: Wilhelm Johannsen and the problem of heredity at the turn of the 19th century *International Journal of Epidemiology* 43(4)*:*1007–1013. doi: 10.1093/ije/dyu066.

Santos C.A.F. and Boiteux. L.S. 2013. Breeding biofortified cowpea lines for semi-arid tropical areas by combining higher seed protein and mineral levels. *Genetics and Molecular Research* 12 (4): 6782-6789.

Selinger D.A. and Chandler. V.L. 1999. A mutation in the pale aleurone color1 gene identifies a novel regulator of the maize anthocyanin pathway. *Plant Cell*, 11: 5-14.

Simonne. A. H.. D. B. Weaver. and Wei. C. 2001. Immature soybean seeds as a vegetable or snack food: acceptability by American consumers. *Innov. Food Sci. Emerg. Technology* 1: 289–296. https://doi.org/10.1016/S14668564(00)00021-7.

Stancheva I. Kostadinka N. Maria G. Radka D. Marieta H. Ionita P. Mariana S. Galina P. Efrosina D. Hristo V. 2016. Nutritional value of cowpea (*Vigna Unguiculata* L. Walp) grain grown under different soil moisture as affected to the dual inoculation with nitrogen fixing bacteria and arbuscular mycorrhizal fungi, *Bulgarian Journal of Soil Science*, Volume 1. Issue 2.

Tan H., Tie M., Luo Q., Zhu Y., Lai J. and Li H. 2012. A Review of Molecular Makers Applied in Cowpea (*Vigna unguiculata* L. Walp.) Breeding. *Journal of Life Sciences.* 6: 11901199.

Tejashree S., Lachyan, V. Dalvi, V. 2015, Quantitative Traits in Cowpea (*Vigna unguiculata* (L.) Walp), *International Journal of Science and Research (IJSR)*, Volume 4 Issue 4.

Timko M. P. and Singh B. B. 2008. Cowpea a multifunctional legume. More P. H., Ming R. (eds.), *Genomics of Tropical Crops Plants.* 237- 238.

Yeung H., Ehlers J. D., Waniska R.D., Alviola, J.N., Rooney L.W. 2009. Rapid screening methods to evaluate cowpea cooking characteristics. *Field Crops Research* 112, 245–252.