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Evaluation of Some Pepper Accessions (*Capsicum spp***) Based on Qualitative and Cytological Attributes**

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Abstract:

Field experiment for this research was conducted at Gidan Mangoro village, Bosso local government area of Niger State to evaluate some indigenous pepper accessions such as Dan-Birnin Gwari (DBG), Dan-Zaria 'Tatashe (DZ-TSH), Dan-Kaduna (DKD), Dan-Gada'Shombo' (DG-SB), Dan-Sokoto 'Rodo' (DSKT-RD), Dan-Sokoto 'Tatashe' (DSKT-TSH), Dan Sokoto 'Shombo' (DSKT-SB), Dan Kano (DKN), Dan-Adamawa (DADAM) and Dan-Katsina (DKST). The treatments were laid down in a randomized complete block design with three replications. The cytological investigation was carried out at the laboratory of the Department of Crop Production, Federal University of Technology, Minna, Niger State. The treatments were laid down in Randomized Complete Block Design with three replications. The plot size was 13 m \times 25.5 m(331.5 m²). Sowing of seed at nursery took place in April at the onset of the rain while the seedlings were transplanted into the field at six weeks after sowing. Data collected based on qualitative traits were as follows; plant growth habit, leaf shape, corolla colour, fruit colour, fruit attitude and fruit cross-section, anthocyanin colouration of nodes, calyx constriction Principal Component Analyses were carried out with statistical means and mode of each variable. Cluster analyses were determined using the unweight pair group method with arithmetic mean (UPGMA) and the accessions were categorized into three groups. Correlation coefficients of qualitative traits were also determined. The cytological study revealed that at meiosis, two daughter cells divides and formed four daughter cells thereby contributing to genetic variation. The study revealed the phenotypic variations that existed among the ten pepper accessions and how these variation could importantly be useful in the crop breeding and improvement programme. The study was conducted to provide a reliable passport data for easy accessibility and for subsequent selection for breeding work. Percentage distribution in anthocyanin coloration showed that all the ten accessions studied possessed this coloration in their nodes while for fruit pigmentation, only few accessions have the traits. Clustering of accessions were based on genetic similarities as those found to be genetically related are all grouped into similar cluster as oppose to those who show phenotypic divergence. Correlation studies revealed the mutual relationship and how some of the observed traits can best blend in term of crosses. The chromosomes behaviours during the study showed how qualitative traits are transmitted from one generation to another. Based on the findings of the research work, it is recommended that breeders should collect germplasm from DKD-RD and DG-SB, for further breeding and improvement programme of the crop.

Keywords: Genetic variation, qualitative traits, cytological behaviours

1. Introduction

Pepper belongs to the genus *Capsicum* and member of the nightshade family *Solanaceae*. This genus also called the chilli pepper (Joshi, 2012) originated from Central and South America (Grubben and El-Tahir, 2004). There are about thirty species in the genus *Capsicum* and several species have been domesticated to produce many cultivated types which range from mild to hot (Bosland and Votavas, 2000). Fruit characters have been extensively used in the taxonomy of the family Solanaceae (Pabon-Mora and Litt, 2011).Most natural populations of pepper are diploid and have the same chromosome number, 2n=24 (Grubben and El-Tahir, 2004; Okoli and Osuji, 2008). Nigeria is known to be one of the major producers of pepper in the world accounting for about 17 % with 78,462 t from 90,000 ha of the world production (Food and Agriculture Organization, 2013). Chillies are largely grown in many parts of Nigeria and the major area of production is the northern region between latitude 10° N and 12° 30'N (Adetula and Olakojo, 2006). The chilli peppers are excellent source of nutrition for humans, a rich source of most B vitamins and vitamin B₆ in particular (Deepa *et al.*, 2007; United SDA Nutrient Database, 2007). They can be eaten raw, cooked or processed into powder form for culinary uses (Collingham, 2006).

Estimating the amount of genetic variability and determining the nature of the association between variables are very important steps in selection and improvement programme. Furthermore, the success of any selection programme depend largely on the amount of genetic variability existing in the population. Cytological and agro-morphological traits have been found to provide a good assessment of variability in *capsicum* species (Del *et al.*, 2007; Bozokalfa *et al.*, 2009).

2. Materials and Methods

2.1. Experimental Site

The research was conducted at Gidan Mangoro village, Bosso Local Government Area of Minna, located at an elevation of 482 meters above sea level in the Southern Guinea Savanna zone of Nigeria and lying between latitude 9° 33′57.69′N, longitude 6° 29′19.896′E. The elevation of the site was tracked using GPS (Geographical Positioning System, Garmin Taiwan). The Cytological investigation was carried-out at the Laboratory of the Department of Crop Production, Federal University of Technology Gidan Kwanu Campus, Minna, Niger State of Nigeria.

2.2. Treatment and Experimental Design

Ten accessions of chilli pepper collected from local farmers in different locations of Northern Guinea Savanna and Sudan savanna of Nigeria were evaluated in this study. Seeds of four accessions were collected from Kaduna State [Dan-Birnin Gwari (DBG), Dan-Zaria 'Tatashe (DZ-TSH), Dan-Kaduna (DKD), Dan-Gada' Shombo' (DG-SB)], three accessions Sokoto State [Dan-Sokoto 'Rodo' (DSKT-RD), Dan-Sokoto 'Tatashe' (DSKT-TSH) and Dan Sokoto 'Shombo' (DSKT-SB)], one from Kano State [Dan Kano (DKN)], one from Adamawa State [Dan-Adamawa (DADAM)], and one from Katsina State [Dan-Katsina (DKST)]. The treatments were laid down in Randomized Complete Block Design with three replications. The plot size was $13 \text{ m} \times 25.5 \text{ m}(331.5 \text{ m}^2)$. Sowing of seed at nursery took place in April at the onset of the rain while the seedlings were transplanted into the field at six weeks after sowing.

2.3. Data Collection and Statistical Analysis

Growth habit, leaf shape, corolla colour, calyx annular constriction, anthocyanin colouration of node, fruit colour at immature stage, fruit colour at maturity, fruit shape at maturity, fruit attitude, fruit pigmentation, fruit pedicel colour at maturity, fruit cross-section (at placenta level). All the data collected were subjected to cluster analysis, principal component analysis and correlation analysis.

3. Results

3.1. Cluster Groupings of the Accessions Based on Qualitative Traits

There were three clusters presented in Figure 1.0. Cluster 1, comprises DSKT-RD and DKD-RD which showed 13.58 % similarity, while cluster 2, consisted of DSKT-TSH, DG-SB, DADAM, DZ-TSH, DKN, DSKT-SB, and DBG with 73.32 % similarity. However, within the cluster there were about six sub-groups. Also cluster 2 consisted of two sub-clusters: Sub-cluster 1 consisted of DADAM and DZ-TSH, which are closely related accessions. This sub-cluster was joined by DG-SB, to form a sub-cluster, while the next hierarchy was followed by the association of DSKT-TSH. The second sub-cluster also existed within the same cluster (cluster 2) and this sub-cluster consisted of DSKT-SB and DBG, which also showed a closer relationship as in the case of sub-cluster 1. This sub-cluster was joined by DKN followed by the inclusion of DSKT-TSH, next on the hierarchy was DG-SB and finally linked back to the first sub-cluster (DADAM and DZ-TSH). Cluster 3, consisted of only one outlier accession: DKST which showed no cluster with any other accession (that is, it is distantly related to all the other accessions in terms of grouping).

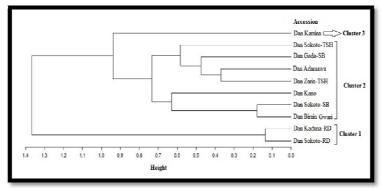


Figure 1: Dendogram of the Ten Pepper Accessions Inferred from Qualitative Traits Using Unweighted Pair Group Method With Arithmetic (UPGMA) Means

3.2. Principal Components Based on Qualitative Traits

Results of principal component analysis for qualitative traits are presented in Table 1. This indicated that five principal component axes existed. These axes; PCA1, PCA2, PCA3, PCA4 and PCA5, contributed 35.99, 29.03, 17.32, 9.40, 82.34 and 5.50 % to the total variation respectively. The result showed that immature fruit colour (0.562), fruit pigmentation (0.480), growth habit (0.364), pedicel colour (0.193) and corolla colour (0.152) differentiated the traits on the first principal component axis. The second principal component showed loading on traits such as fruit attitude (0.609), fruit pigmentation (0.372), corolla colour (0.141), fruit shape (0.072) and immature fruit colour (0.045) which differentiated the axis. Third principal component was accounted for by corolla colour (0,713) mature fruit colour (0.642) and growth habit (0.007). Growth habit (0.674), fruit shape (0.537), fruit attitude (0.371), mature fruit colour (0.232) and immature fruit colour (0.154), differentiated accessions at the fourth principal component axis. The fifth principal component axis revealed that traits such as corolla colour (0.626), growth habit (0.219), pedicel colour (0.161) and fruit shape (0.160) differentiated the remaining traits.

Traits	Prin1	Prin2	Prin3	Prin4	Prin5	
GHBT	0.364	-0.299	0.007	0.674	0.219	
CC	0.152	0.141	0.713	-0.050	0.626	
CALC	0.000	0.000	0.000	0.000	0.000	
ANTHOCOL	0.000	0.000	0.000	0.000	0.000	
IMFCOL	0.562	0.045	-0.038	0.154	-0.286	
MFCOL	-0.158	-0.243	0.642	0.232	-0.635	
FSHP	-0.485	0.072	-0.152	0.573	0.160	
FATT	-0.019	0.609	-0.064	0.371	-0.051	
FPIGMT	0.480	0.372	-0.042	-0.01	0-413	
PEDICOL	0.193	-0.562	-0.222	0.009	0.161	
% variation	35.99	29.03	17.32	9.40	5.500	
Cumulative	35.99	65.02	82.34	91.74	97.24	
variation						

Table 1: Eigen Vectors for Principal Component Axes Using Qualitative Traits of the Ten Pepper Accessions

GHBT= growth habit, CC= corolla colour, CALC= calyx constriction, ANTHCOL= Anthocyanin colouration of nodes, IMFCOL= immature fruit colour, MFCOL= mature fruit colour, FSHP= fruit shape, FATT= fruit attitude, FPIGM= fruit pigmentation, PEDICOL= pedicel colouration

3.3. Biplot of the First and Second Principal Components Based on Qualitative Traits

The result of biplot for qualitative traits is presented in Figure 2. The result showed that, fruit attitude, fruit pigmentation and corolla colour contributed positively to variability. Similarly, fruit shape and immature fruit colour were observed to contribute slightly to variability. Also mature fruit colour, growth habit and pedicel colour were noted to suppressed variability in the pepper accessions studied. However, calyx annular constriction and Anthocyanin colour of nodes showed no contribution to variability.

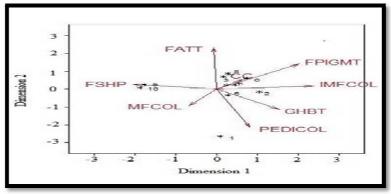


Figure 2: Biplot of the First and Second Principal Components Based on Qualitative Traits of Ten Pepper Accessions

3.4. Correlation Coefficient Matrices for Qualitative Traits

Qualitative coefficient matrixes are presented in Table 2. The result indicated that growth habit was strong and significantly correlated with corolla colour (0.82^{**}) , matured fruit colour (0.88^{**}) and fruit pigmentation (0.52^{**}) . Corolla colour showed a strong positive significant correlation with immature fruit colour (0.67^{**}) , fruit attitude (0.76^{**}) , fruit pigmentation (0.49^{*}) and pedicel colour (0.49^{*}) . Immature fruit colour was significant with fruit attitude (0.78^{**}) , matured fruit colour (0.57^{**}) , pedicel colour (0.42^{*}) and are not-significantly correlated with fruit shape (0.02) and fruit pigmentation (0.003). Association between mature fruit colour with fruit shape (0.78^{**}) showed a high significant correlation, but non-significant correlated with fruit pigmentation (0.22). Fruit shape matrix showed a significant correlation with fruit attitude and fruit pedicel colour (0.45^{*}) , but a non-significant association was observed in fruit pigmentation (0.06). Fruit attitude indicated non-significant correlation between all the traits existing in the matrix; fruit pigmentation (0.14) and pedicel colour (0.01). Fruit pigmentation recorded a highly positive and significant correlation with pedicel colour (0.54^{*}) in this matrix.

Traits	GHBT	CC	CALC	ANTCL	IMFCOL	MFCOL	FSHP	FATT	FPIGM	PEDICOL
GHBT										
CC	0.82**									
CALC	0	0	0							
ANTHCOL	0	0	0	0						
IMFCOL	0.08	0.67**	0	0						
MFCOL	0.88**	0.39	0	0	0.57**					
SHP	0.43*	0.39	0	0	0.02	0.77**				
FATT	0.42*	0.76**	0	0	0.78**	0.39	0.39			
FPIGM	0.52**	0.49*	0	0	0.003	0.21	0.06	0.14		
PEDICOL	0.10	0.49*	0	0	0.42*	1.00	0.45*	0.01	0.54**	

Table 2: Correlation Matrices for Qualitative Traits of the Ten Pepper Accessions Studied

Note: * correlation is significant at 0.05, ** correlation is significant at 0.01 GHBT= growth habit, CC= corolla colour, CALC= calyx constriction, ANTHCOL= anthocyanin colouration of nodes, IMFCOL= immature fruit colour, MFCOL= mature fruit colour, FSHP= fruit shape, FATT= fruit attitude, FPIGM= fruit pigmentation, PEDICOL=

3.5. Cytological Investigation and Findings

The meiotic chromosomes study of the ten pepper accessions are presented in (Figure 3). The result of the cytological study of accession DKN under a light microscope revealed that at diakinenssis, chromosomes are mostly associated with bivalent from the first phase to the last phase of meiosis. At the first stage of meiosis, accession DKST was observed to be at the resting phase (interphase I). In this stage no chromosomes were observed except a thread-like structure (a). Accession DSKT-SB showed a normal segregation pattern of chromosomes at metaphase I, with bivalent aligned at the spindle equator (b). While at the early prophase, chromosomes were observed forming a bivalent as in accession DK-RD and DBG (c). At early anaphase, two bipolar spindles were observed in accession DKN with chromosomes migrating to the poles (d and e). At interkinesis chromosomes were observed in separate spindle dyad cell (g). At late telophase, accession ADAM showed a complete separation of the two sister's cell from each other. Accession DKST at telophase showed an abnormal disjoint forming a triad (f). On the contrary, accession DKN and DG-SB showed a linear and regular tetrad after the disjoint at telophase II (h and i) respectively.

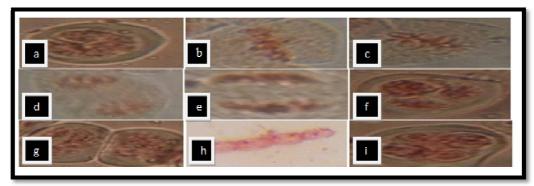


Figure 3: Cytological events: a= Interphase, b= Early metaphase I, c= Late prophase, d=Early anaphase, e= Late anaphase, f= Triad (at telophase), g= Dyad (interkinesis), h= Linear tetrad at telophase, i= Telophase II

4. Discussion

This research revealed that variation existed among the accessions in respect to traits studied. The variability that existed among the accessions were observed in traits such as growth habit, leaf shape, corolla colour, calyx annular constriction, anthocyanin colouration of node, fruit colour at immature stage, fruit colour at maturity, fruit shape at maturity, fruit attitude, fruit pigmentation, fruit pedicel colour at maturity, fruit cross-section (at placenta level), might be due to environmental factor as well as genetically influenced. This is in line with the findings of Del et al. (2007) who reported that, the variation observed in pepper genotypes may be mainly due to the genetic difference among different plants, soil type and environmental conditions of the evaluated site. With respect to flower morphology accession DKN showed a distinctive yellow corolla colouration. The yellow colour observed in this accession differentiated the other nine accessions. This agrees with the work of Sudre et al. (2010) who reported that yellow flower colour and yellow pistilate can be used to differentiate the cultivated pepper from the wild types. Qualitative cluster analysis revealed that three discriminate groups existed in the studied accessions and each of the clusters contributed to variability. Among these three clusters existed one outlier accession DKN. The outlier showed a variation in corolla colour and number of branches with respect to the other nine accessions and this might be due to its genetic freedom. This agrees with Maga et al. (2012) who reported that when an outlier existed within a genotype it is a confirmation of genetic independent. The four principal component analysis showed that most of the traits studied a positive loading, while others showed a negative loading at all. The high percentage loadings observed in some of the accessions has help in contributing to the total variations. Correlation analysis revealed that most of the accessions studied have strong and significant correlation which might be desirable to breeders for further breeding and improvement of the crop.

At meiosis I, the parent cells segregated into two cells. This agrees with Yu *et al.* (1997) who reported that at meiosis I, one haploid segregate forming two daughter cells. From the result of the meiosis at telophase II, it was also observed that there was an irregular disjunction resulting from chromosomes aberration. This agrees with the findings of Rodriguez *et al.* (2012); Abubakar *et al.* (2015) that chromosomes aberration was due to irregular chromosome segregation at meiosis and can result to unsuccessful transmission of some important traits.

5. Conclusion

The study revealed the phenotypic variations that existed among the ten pepper accessions and how these variation could importantly be useful in the crop breeding and improvement programme. The study was conducted to provide a reliable passport data for easy accessibility and for subsequent selection for breeding work. Percentage distribution in anthocyanin coloration showed that all the ten accessions studied possessed this coloration in their nodes while for fruit pigmentation, only few accessions have the traits. Clustering of accessions were based on genetic similarities as those found to be genetically related are all grouped into similar cluster as oppose to those who show phenotypic divergence. Correlation studies revealed the mutual relationship and how some of the observed traits can best blend in term of crosses. The chromosomes behaviours during the study showed how qualitative traits are transmitted from one generation to another.

6. Recommendation

Based on findings from this research, it is recommended that breeders should collect germplasm from DSKT-RD, DADAM and DKD-RD for further breeding and improvement programme due to high presence of anthocyanins which might be useful in providing attraction for pollination and can also be targeted for disease resistant in the crop. Farmers should also be encouraged to patronize improved seeds from these accessions to enhance good production.

7. References

i. Abubakar, A., Falusi, A. O., Daudu, O. A. Y., Oluwajobi, A.O., Dangana, M. C & Abejide, D. R. (2015). Mutagenic Effects of Sodium Azide and Fast Neutron Irradiation on the Cytological Parameters of M2 Lagos Spinach (*Celosia argentea* var *cristata* L.) *World Journal of Agricultural Research*, 2015, Vol. 3, No. 3,107-112Available online at http://pubs.sciepub.com/wjar/3/3/3. Science and Education Publishing. DOI:1012691/wjar-3-3-3.

- ii. Adetula, A. O & Olakojo, S. A. (2006). Genetic Characterization and Evaluation of Some Pepper Accessions *Capsicum frutescens* (L.): The Nigerian 'Shombo' Collections. *American-Eurasian Journal of Agricultural and Environmental Science*, 1 (3): 273-281.
- iii. Bosland, P. W & Votava, (2000). *Peppers; vegetable and spicecapsicums*. CABI Publishing, Wallingford, United Kingdom. Pp. 204.
- iv. Bozokalfa, M. K., Esiyok, D., & Turhan, K. (2009). Petterns of phenotypic variation in a Germplasm collection of pepper (*Capsicum annuum* L.) from turkey. *Spanish Journal of Agricultural Research*, 7(1): 83-95.
- v. Collingham, E. (2006). Curry. Oxford University Press. ISBN 0-09-943786-4.
- vi. Deepa, N., Kaur, C., George, B., Singh, B., & Kapoor H.C. (2007). Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity. LWT- *Food Science Technology*. 40(1):12-129.
- vii. Del, E., Moreno, P. C., Cruz, A. O., Avendano, A. C. H., Martinez-Damian, M. A. T & Pena, L. A. (2007). Morphological variation in Guajillo chilli pepper plants (*Capsicum annuum* L.). *African Crop Science* Conference. Volum 8. Pp. 327-332.
- viii. Duncan, E. B. (1955). Multiple Range test and multiple F-tests. *Biometrics*, 11: 1-42.
- ix. FAOSTAT (2013). Food and Agricultural Organization of the United Nations Statistical Database, Rome, Italy.http://faostat.fao.org/site/339/default.aspx.
- x. Grubben, G. J. H & El-Tahir, I. M. (2004). *Capsicum annuum* L. In: Grubben, G. J. H., and Denton O. A. (Eds.). PROTA 2: *Vegetables/Legumes. [CD-Rom]. PROTA*, Wageningen, The Netherlands.
- xi. Joshi, M. (2012). Chilli Pepper Institute studies what's hot. *Your life* (USA Today). Archived on (12th March, 2012).
- xii. Maga, T. J., Uguru, M. I & Ogbonna, P. E. (2010). Pattern of genetic variability in Nsukka yellow pepper (*Capsicum annuum* L.) *Journal of Agricultural Research*. Pp 77-94.
- xiii. Okoli, B. E & Osuji, J. O., (2008). The Status of Research on the Cytogenetic of Nigerian Fruit Crop. *Nigerian Journal of Botany* 21[2]; 358-372.
- xiv. Pabon-Mora, N & Litt, A. (2011). Comparative anatomical and developmental analysis of dry and fleshy fruits of Solanaceae, *American Journal of Botany*, 98 (9), Pp. 1415–1436.
- xv. Rodriguez, V. M., Rodriguez-Garay, B & Barba-Gonzalez, R. (2012). Meiotic restitution mechanisms involved in the formation of 2n pollen in (*Agave tequilana* Weber and *Agave angustifolia* Haw. Springer Plus), 1(17), 1-7, 2012. Available online athttp://www.springerplus.com/content/1/1/17. (Accessed on 27th August, 2014).
- xvi. Sudre, C. P., Goncalves L. S. A., Rodrigues, R. Amaral- Junior, A. T., Riva-Souza, E. M & Bento, C. S. (2010). Genetic variability in domesticated *Capsicum* spp. As assessed by morphological and agronomic data in mixed statistical analysis. *Genetics and Molecular Research*. 9: 283-294.ISSN 1676-5680.
- xvii. United State Department of Agriculture Nutrient Database (2007). Retrieved from http://www.ars.usda.gov/nea/hrc/n