



GENOTYPE-ENVIRONMENT INTERACTIONS AND YIELD STABILITY OF COWPEA (*Vigna Unguiculata L. Walp*) IN LOMAMI PROVINCE, CENTRAL PART OF DEMOCRATIC REPUBLIC OF CONGO

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ABSTRACT

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The genotype environment interaction (GEI) has always been considered as an important issue by researchers involved in varietal selection and yield performance assessment. The objective of this study was to identify stable genotypes of cowpea in the Greater Kasai region. The current study was undertaken in 3 locations in the Ngandajika territory of Lomami Province (central part of Democratic Republic of Congo (DRC) with 12 improved cowpea genotypes obtained from the National Institute for Agricultural Research and Studies. The experiments were conducted following the randomized block design with 3 replicates and 12 treatments. Analysis of variance (ANOVA) and AMMI model were applied to identify main effects and GEI. The genotype yield stability across different locations was determined using the stability value of AMMI (ASV) rank of the AMMI model. ANOVA test indicated that there was performance inconsistencies in the yield of the 12 genotypes tried across the environments retained in this study. However, AMMI model revealed genotypes with stable yields across the different environment considered in the study. For the determination of the specificity of adaptability of genotypes to specific environments, the stability value of AMMI and diagram plot techniques were used for the discrimination of the genotypes versus the different environments considered. The genotypes Mujilanga (V2), CNGKASA7-2-M (V3) and CNGKASC2-1-1-T (V9) were identified as the most stable across different environments studied. Their yields were significantly higher ($P < 0.05$) since they oscillated between 260 and 369 kg / ha as compared to the overall average of 312 kg / ha.

Contribution/Originality: This study is one of very few studies which have investigated yield stability and adaptability of cowpea genotype in central part of DR Congo. The paper's primary contribution is finding that stability value of AMMI (ASV) rank and diagram plot discriminated three cowpea genotypes for dissemination in central part of DR Congo.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important legume crop grown in tropical areas of Africa, Asia and America. Globally, the crop is grown on about 12.7 million hectares for its production (6.4 million tons) (Nteranya and Bergvinson, 2015). In the Democratic Republic of Congo (DRC) the crop is grown on nearly 149.305 hectares with an annual production of about 77604 tons (INS, 2015).

Cowpea is grown across almost the 26 provinces of the DRC. About 47.9% of the national production comes from the province of Lomami alone (Mukendi *et al.*, 2017).

This legume crop is much appreciated by local communities. The crop is the key source of vegetable proteins for the population. Cowpea crop plays a key role in the formation of rural income of the population. The crop is also a key source of income; thus it plays a key role in the food security strengthen of the community (Kadima, 2006; Dwivedi *et al.*, 2015).

However, cowpea production is generally constrained by several biotic and abiotic factors in Central DR Congo. There are scientific evidence that the absence of improved high-yielding genotypes, soil infertility, uneven rainfall distribution (climate change and variability), prevalence of diverse diseases, pests and parasitic plants, are among key limiting factors of cowpea production and productivity (Dolinassou *et al.*, 2016; Lydia, 2016). The sensitivity of genotypes to different environmental conditions is also a key cause of the fluctuation in the annual production of the cowpea crop (Sadeghi *et al.*, 2011). To break with these production constraining factors, researchers are seeking for varieties that are tolerant, locally adapted with low susceptibility to biotic and abiotic constraints in order to minimize or reduce on the risk related to yield loss (Sadeghi *et al.*, 2011). Currently, breeders are designing climate smart-genotypes that are likely to provide optimal yields despite the multiplicity of limiting biotic and abiotic factors (Bose *et al.*, 2014). The scaling-up and wide dissemination of climate-smart genotype may be an effective option for less resourced small scale farmers. It is likely that improved genetic material that is tolerant to abiotic and biotic stresses may be adopted quickly by farmers, provided that socio-economic and market characters are imbedded within the different varieties that are proposed. Currently, there are only 10 genotypes available in the national plant genetic resources program. These 10 cowpea varieties were selected 15 years ago by local breeders. In the current context of the changing (variability) of biotic and abiotic factors, the paucity of the INERA Ngandajika cowpea gene bank, the lack of access to improved and adapted varieties, the absence of organized seed market system in the region, are among key constraints for the expansion of cowpea production in other provinces of DR Congo.

Some adaption measures have been developed by producers such as self-generation of seeds for some varieties. Unfortunately, the material used by farmers is composed of varieties with low yield potential. Currently, breeders face the challenge of availing pest-tolerant and high yielding and adapted varieties to producers.

In this regards, there is a need for researchers to select and disseminate genotypes with multiple attributes including tolerance to pests and diseases and high adaptability to local environmental characteristics. It is likely that such important work may start by the recovery, homogenization, and purification of different varieties from the local gene bank. It is important to identify site specific adaptation characteristics and select varieties that have expressed or shown optimal and regular yields across various growing places (Rojas-Downing *et al.*, 2017). Farmers need varieties likely to provide acceptable yield under favorable and unfavorable environmental conditions. Available genotypes at the research station are susceptible to pests (thrips, bugs, borers, aphids, etc.) and diseases and it is difficult to currently recommend them to the advisory (extension) services for large dissemination.

Researchers need to know the environmental response of different genotypes before releasing them for dissemination (Simion *et al.*, 2018). Breeders are aware that different improved genotypes express different levels of sensitivity and performance from one environment to the other. Quiet often, genotypes are affected by the phenomenon of genotype x environment interaction (GEI), meaning that behavioral response of varieties is shaped by environmental characteristics. Under GEI conditions, for a genotype with high potential, there is variability in

its yield components and the yield performance is mostly driven by local environmental characteristics (Singh. *et al.*, 2015). It is the interaction of environment that causes irregularity in yield performance of different genotypes from year to year and from place to place (Akter *et al.*, 2015; Owusu *et al.*, 2018).

For this reason, it is common practice to evaluate genotypes in space and time by testing the degree of interaction for environmental adaptation by various genetic materials. Overall, a genotype is considered to be adapted or stable if its yield is moderately high while expressing a small fluctuation of its yield capacity (Dolinassou *et al.*, 2016).

Stability, on the other hand, is the ability of a genotype to always have consistent yield performance without worrying about the negative effects of environmental variability. Information on the effect of genotypes, the environment and their interactions on yield of legume crop (including cowpea) is not available across the different agro-climatic conditions within DRC. Not only there is lack of information on climate-smart genotypes but also breeding programs dedicated to the development of such varieties do not exist in the country (DR Congo). There is a need for developing specific breeding research program to address these crop productivity and food security issues. There is a need also to reduce on the knowledge developmental practice gaps.

There is an urgent need to identify genotypes with broad or specific adaptation and stability across years and sites (environments). This is feasible by using multi-year and location design protocols. This approach is likely to increase the likelihood of genotype adoption by producers as well as making suggestion (way forward) for future improvement work. Thus, scientific work aiming at identifying improved genotype with stable yield and adaptation to specific environment, require special protocol designs. In fact, researchers aiming at identifying environmental behavior of different genotypes often use specific technique such as “Genotype x Environment Interactions”, during data analysis process. Various approaches during GEI tests are based on the analysis of variance derived from regression (Zobel *et al.*, 1988; Patel *et al.*, 2015; Noerwijati *et al.*, 2017). For example, factorial regression seeks to decompose the effect of the environment by covariates that are most often environmental and that are often considered as factors that explain variations in yield (Ding *et al.*, 2008).

However, although GEI method is widely used over the last 20 years, there are limitations mainly, notably by not taking into account of correlations between variables (Das *et al.*, 2011; Sabaghnia *et al.*, 2013). Hence, to characterize general adaptation of genotypes to specific environments, ANOVA, joint regression, factorial regression and bi-additive factorial regression models are selected depending on the number of environmental factors or constraints that are considered to identify characteristics of specific adaptation of genotypes.

Several studies related to analysis for stability and genotype environment interaction in plant breeding are available today; they are complementary to each other and focus on different approaches (techniques) depending on the authors. The technique of joint regression according to Finlay & Wilkinson model (Rodrigues *et al.*, 2011) using the estimates of the regression of the yield of each variety as compared to the index of the medium (I) which is the difference of the average of the genotypes in a place (Y_j) deduced from the large mean (Y) such as:

The regression equation is: $Y = b I + a$ where $I = Y_j - Y$.

This technique (non-parametric model) has been applied by researchers aiming at exploring adaptability of 20 durum wheat genotypes. This technique uses the rank of genotype yield to explain GEI (Karimzadeh *et al.*, 2013). In addition, Eberhart and Russell model was used to identifying response of 17 sesame genotypes in 4 different environments. The model quietly defines the regression coefficient (b_i) and the regression mean deviation square (S^2_{di}) to explain the partition of genotype, environment, and GEI variation (Yirga, 2016). Rodrigues *et al.* (2011) used a model based on the Finlay Wilkinson regression protocol to analyse data that was collected from multi-environment trial designs. The analysis aimed at determining the additive main effects. The analysis used multiplicative interaction (AMMI) model with particular focus on robustness by increasing amounts of randomly selected missing data.

Quiet often, approaches used by above mentioned authors enabled to identify individual characteristics of stability but did not provide an overall picture of the varietal response to various environments. Therefore, a single parameter is not sufficient to characterize the stability of a genotype because several traits are often associated with yield response to specific environment; hence the need to conduct more complex analyses (Mukherjee *et al.*, 2013) Multiplicative models that allow a richer interpretation such the additive effects and the Multiplicative Interaction Model (AMMI) are more robust multi-variate method for multi-environmental trial assessment. The AMMI method is classically applied to assess varietal responses and yield behaviors during multi-trial designs.

Ecovalence appears as a synthetic and robust relative indicator to quantify the level of interactivity of varieties and places. Varieties that are very sensitive to the environment always appears to have strong ecovalences, whereas varieties that are more stable or have a behavior, with each pedo-climatic condition encountered, that have yields that are very close to average of all varieties, are generally considered as varieties with low ecovalence (Kilic *et al.*, 2010).

Hence, in this study, it was judged necessary to apply this robust method using the model of additive effects and multiplicative interaction (AMMI) on 12 cowpea genotypes existing at the level of the production zone in the Ngandajika territory of central DR Congo. This research was undertaken to identify response of 12 cowpea genotypes to different environmental conditions that are representative of main agro-ecological zones where cowpea is grown by farmers in Ngandajika territory. Overall, the study aimed at identifying the most suitable genotypes for Ngandajika area.

The study therefore attempted to provide answer to the following question: are there stable genotypes that can produce in various or specific environments from the National genebank found at Ngandajika Research station?

The main objective of this study was to:

Contribute to identification of genotypes with high yield stability to local environments.

The specific objectives were:

- (i) To determine the yield stability of 12 cowpea genotypes across different localities of Ngandajika territory (Lomami province), using AMMI models;
- (ii) To assess the productivity of the 12 genotypes in all three environments of Ngandajika territory (Lomami Province, central DR Congo) and identify the most productive ones to be released in rural areas

Hypotheses of the study were:

- (i) Genotypes with greater stability and adaptability across different agro-ecological zones are likely being adopted by farmers;
- (ii) Cowpea productivity in Lomami province may be linked to intrinsic genotypic potential of material

This paper is structured as follows: introduction, material and methods, results and discussion.

2. MATERIAL AND METHODS

2.1. Study Area and Experimental Sites

2.1.1. Study Area

This study was conducted in Ngandajika territory (Lomami Province) during the cropping season B (January-May) of year 2016. Ngandajika territory (6 °45'00"S, 23°58'01"E) is bordered by the territories of Kabongo in the East, Tshilenge across river Luilu in the West, Katanda and Kabinda in the North, and Luilu in the South. Ngandajika is located 90 km far from Mbuji-Mayi town. The territory is found at about 130 km far from Kabinda town.

Since colonial time, Ngandajika territory is known to be home of prosperous agriculture practiced by the Bantous people. The largest tribe among the Bantus found in Ngandajika territory is the Luba tribe.

Ngandajika area is covered by the Guinean savanna that is dominated by *Hypparennia* and *Imperata cylindrica* grasses.

The territory of Ngandajika is covered with ferralsols. These soils are characterized by a collection of sandy sediments with heavy clay. The soil of Ngandajika is a sandy-clay texture, but there are particularly clay soils often encountered along the rivers. The soils of Ngandajika are also influenced by microclimate of forest galleries found here and there. Practically, the soils are generally of red color, these soils are characterized by an average structure found most often on an old lateritic roc (Muyayabantu *et al.*, 2012). These soils are generally acidic and belong to the group of ferralsols according to the global referential base of soil classification (Mukendi *et al.*, 2017). Soil analysis results for INERA and Mpiana sites are published by Muyayabantu *et al.* (2012).

The territory of Ngandajika has enormous hydropower potentials. There are many rivers flowing through such as the Lubilanji River. However, river flow volumes are influenced by erratic rainfall.

Ngandajika society is heterogeneous. Most people (85%) practice agriculture and about 15% of the population is busy with other kind of livelihood and income generating activities such as (teachers, leaders, officers, manpower for agriculture and labour).

The climate of the area has been classified as the Koppën AW4 type. This climate is characterized by a long rainy season (August–May) and a dry season (June–August). The average daily temperature is about 25.3°C and the average annual precipitation is of about 1500 mm.

There are numerous tribes and languages in the Ngandajika territory, but the Luba tribe dominates. In 2014, the total population was of about 1.388.408 individuals living on a total area of 5726 square kilometers.

The main crops grown by farmers include cassava, maize, paddy rice, groundnut, cowpea, soya bean, voandzou, millet, sweet potato. Among livestock activities, farmers are practicing fish farming and livestock keeping. Crop and livestock productions are marketed in main cities such as Mbuji-Mayi town (the Capital of Kasai Province).

2.1.2. Experimental Sites

Research station of INERA

On-station research experiment was conducted at the Agronomic Research station of Ngandajika of the National Institute for the Study and Agricultural Research (INERA). Ngandajika Research station (6°45' South Latitude, 23°57' East longitude with an altitude of 790 m) is administratively located in Ngandajika territory, Lomami Province, central DR Congo [Figure 1](#). Ngandajika research station is covered by a soil with the following soil characteristics: 5.16 of pH, 0.084% of nitrogen (NO_3^-), 0.25 mg/ kg of phosphorus (P_2O_5), 9.62 mg/ kg of K_2O , 9.2 mg/kg of Mg and 10.1 mg/ kg of Fe.

On-Farm Experiments

On-farm experiments were conducted during the cropping season B (January–May) of year 2016 at Yamba and Mpiana village field sites. These two sites are found in the periphery of the INERA Ngandajika research station [Figure 1](#). The Mpiana site (latitude: 6°36'S, 23°54' E, altitude: 685 m) is located in the village plantations at about 28 km from the Ngandajika research of INERA. Soil at Mpiana site has the following characteristics: 5.38 of pH, 0.084% nitrogen (NO_3^-), 0.06 mg/ kg of phosphorus (P_2O_5), 26.4 mg/kg of K_2O , 42.5 mg/kg of Mg and 1.00 mg of Fe.

The Yamba site (latitude: 6°46 'S, longitude: 24°1', altitude: 700 m) is located at about 11 km far away from the Ngandajika research station.

These two field sites receive different rainfall levels and are characterized by different levels of soil fertility and natural vegetation (*Imperata cylindrica*, *Hypparrhenia rufa* vegetation type). However, farmers from these two field sites use low yielding and pest-sensitive genotypes. In these field sites, cowpea is widely grown and there exist a commercial seed market although varieties marketed are sensitive to abiotic and biotic stresses.

These two field sites were selected since they are major cowpea growing areas. In addition, farmers from these areas are more cooperative with extension services; they are likely accepting and adopting quickly innovative

technologies that may be disseminated by researchers and extension services. Farmers from these areas are likely impacting positively on other farmers from the surrounding since they are quick adopters of new information delivered by extension services.

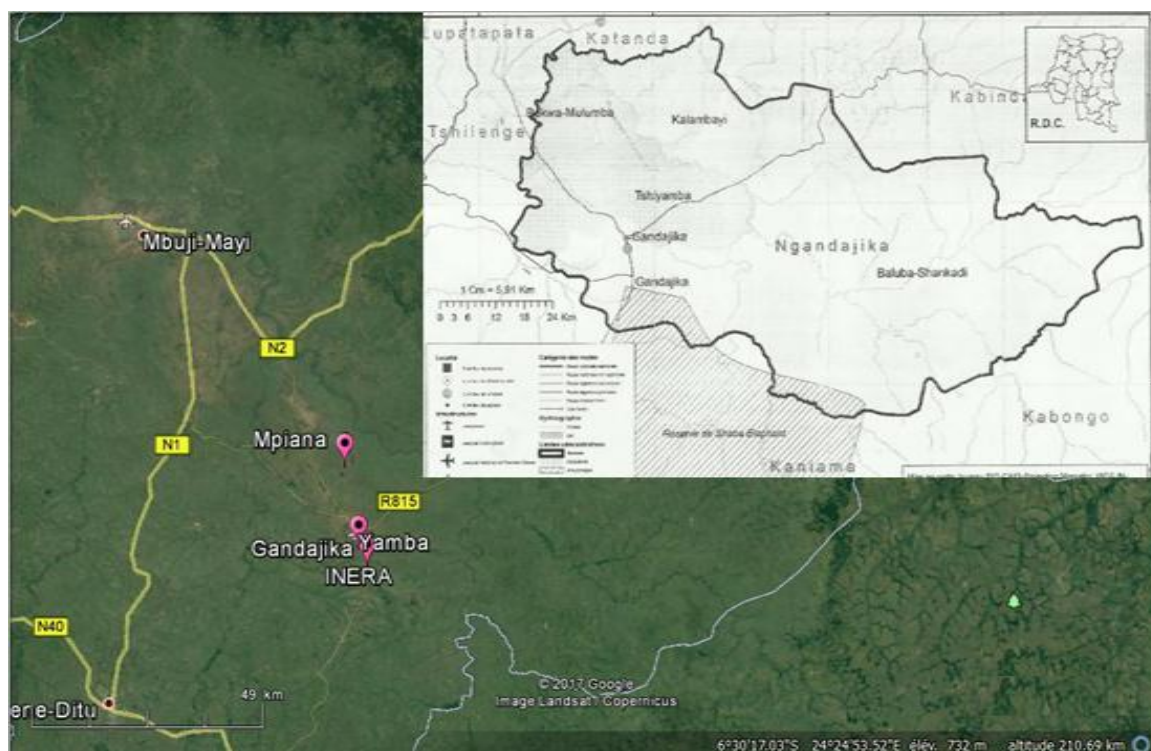


Figure-1. Map of Lomami province showing study locations (Ngandajika, Mpiana, Yamaba sites).

2.2. Experimental Designs

At both on-station and on-field sites, complete block randomized designs (RCBD) were applied. Each RCBD had 3 replicates and 12 treatments (cowpea varieties). Each plot was of 3m x 2.4m wide. The 12 cowpea genotypes (CNGKASC2-1-1-T, CNGKASC6-1-L, CNGKASE2-0-T, Mujilanga CNGKASC5-1A-M, CNGKASH1-1-M, CNGKASB5-2-0-T, CNGKASA2-2- L, Diamond, Yamashi CNGKASG1-0-T CNGKASA7-2-M) were supplied by INERA, Ngandajika Station Table 1.

Table-1. Characteristic of 12 cowpea genotypes studied.

| Code | Genotype | Origin | Growth habit | Maturity | Texture | Weight 100 seed(g) | Yield (Kg/ha) |
|------|----------------|----------|-------------------------|----------|---------|--------------------|---------------|
| V1 | Yamashi | Nigeria | Partly-linear | Meduim | Rough | 11 | 800 |
| V2 | Mujilanga | Tanzania | Partly linear | Meduim | Rough | 11 | 800 |
| V3 | CNGKASA7-2-M | RD Congo | Erected (linear) | Meduim | Rough | 12 | 950 |
| V4 | CNGKASA2-2-L | RD Congo | Erected (linear) | Meduim | Rough | 12 | 1100 |
| V5 | CNGKASG1-0-T | RD Congo | Erected (partly-linear) | Meduim | Rough | 13 | 850 |
| V6 | CNGKASC5-1A-M | RD Congo | Erected (linear) | Meduim | Rough | 12 | 1300 |
| V7 | CNGKASH1-1-M | RD Congo | Erected (linear) | Meduim | Rough | 11 | 1250 |
| V8 | CNGKASC6-1-L | RD Congo | Erected (linear) | Meduim | Rough | 11 | 1200 |
| V9 | CNGKASC2-1-1-T | RD Congo | Erected (linear) | Meduim | Rough | 12 | 1200 |
| V10 | CNGKASE2-0-T | RD Congo | Erected (linear) | Meduim | Rough | 11 | 1000 |
| V11 | Diamant | RD Congo | Partly-climbing | Early | Rough | 12 | 1000 |
| V12 | CNGKASB5-2-0-T | RD Congo | Erected (linear) | Meduim | Rough | 13 | 1100 |

Cowpea was sown (3 seeds per hole) at a spacing of 60 cm x 20 cm apart in the plot. Overall, each experimental plot was covered by about 83,333 plants / ha. Soil preparation was done with a hand hoe. No fertilizers were applied. Also phytosanitary measures were taken against insect pests.

2.3. Statistical Data Analysis

The yield data of different genotypes was analyzed statistically. ANOVA was applied to detect genotypes and environment effects and their interactions. The genotype effect was considered as fixed while the effect of the environment was considered as random during the analysis process. The differences among varieties and environments were assessed using the least significance mean difference at $P \leq 0.05$.

The adaptability and phenotypic stability analyses were performed by applying AMMI technique as hybrid statistical model (Zobel, 1990) as follow:

$Y_{ge} = m + ag + be + \sum_{n=1}^N \ln_{ggn} \text{den} + r_{ge}$; $n = 1 (1)$; where, g is the genotypes, e = environments; Y_{ge} is the yield of genotype g in its environment e ; m is its great average; ag is the mean deviations of the genotype, be is the average deviation of the genotype in the environments considered, N is the number of EPCAs (essential component P interaction with an axis) preserved in the model; ln being the singular value for the IPCA axis n ; ggn being the values of the eigen vector of the genotype for the n axis, den the eigen vector values of the environment for the IPCA axis, n and r_{ge} being the residuals.

$(G \times L)_{ij} = \sum_{n=1}^N \ln_{uni} v_{nj} + r_{ij}$ where \sum is the sum of the $n = 1, 2, n$ axes of the principal component analysis (PCA) integrating the model, ln being the eigenvalue of the axis n , uni being the eigenvector of the genotypes i on the n axis, v_{nj} is the eigenvectors of the site j on the n axis, r_{ij} is the residual of the interaction.

The analyses were done with software R 3.5, using the Factominer procedure which simultaneously gives the results of the ANOVA and AMMI analysis.

The stability value of AMMI (ASV) develops a value of quantitative stability to classify genotypes through the AMMI model. This value has been considered to be the only most appropriate method of describing the stability of genotypes (Naroui *et al.*, 2013).

The ASV model was calculated as recommended (Purchase *et al.*, 2000): with

$$ASV = \sqrt{\left[\frac{SS_{pca1}}{SS_{pca2}} (G_{pca1} \text{ score}) \right]^2 + (G_{pca2} \text{ score})^2} \quad (2)$$

Where SS_{pca1} / SS_{pca2} is the weight given to the IPCA value by dividing the sum of the IPCA1 squares by the sum of the IPCA2

Score G_{pca1} = Score PCA1 score for this specific genotype, and

G_{pca2} = Score PCA 2 score for this specific genotype.

In ASV, genotypes with the lowest ASV scores are considered as the most stable from one environment to another (Purchase *et al.*, 2000; Odewale *et al.*, 2013; Bose *et al.*, 2014).

Performance Stability Index (YSI): The stability index is normally applied to yield data and is referred to as the YSI. This stability index was calculated for each genotype by summing the overall average performance ranking for each trait and the ASV ranking for each trait. YSI was calculated as suggested (Bose *et al.*, 2014).

$YSI = RASV + RY$ (3) where, RASV is the rank of the AMMI stability value and RY is the rank of the average grain yield of the genotypes in all environments. YSI incorporates both average yield and stability into one criterion. The low values of the parameter show desirable genotypes with higher yield.

3. RESULTS AND DISCUSSION

3.1. Genotype Effects

The analysis of the variance showing the effect of genotypes in each site as recorded is given in the table below.

Table-2. Average grain yield (Kg / ha) of 12 genotypes in three environments studied.

| Varieties (Genotypes) names | Experimental sites (Environments) | | | | | | Overall | |
|-----------------------------|-----------------------------------|------|----------------------|------|---------------------|------|---------------|------|
| | INERA site | | Mpiana site | | Yamba site | | Mean | Rank |
| | Mean ± SE | Rank | Mean ± SE | Rank | Mean ± SE | Rank | | |
| Var 2 (Mujilanga) | 393.47 ± 33.6 | 6 | 425.30 ± 151.9 | 6 | 118.72 ± 32.2 | 5 | 315.34 | 6 |
| Var 9 (CNGKASC2-1-1-T) | 472.20 ± 147.3 | 5 | 451.10 ± 72.9 | 5 | 185.13 ± 57.0 | 1 | 369.47 | 4 |
| Var 3 (CNGKASA7-2-M) | 293.93 ± 111.9 | 7 | 377.57 ± 86.6 | 9 | 109.90 ± 20.8 | 6 | 260.46 | 9 |
| Var 12 (CNGKASB5-2-0-T) | 246.67 ± 2.02 | 9 | 255.87 ± 45.8 | 12 | 92.53 ± 11.5 | 12 | 198.35 | 11 |
| Var 11 (Diamant) | 497.67 ± 64.8 | 4 | 360.17 ± 100.1 | 10 | 138.83 ± 36.1 | 3 | 332.22 | 5 |
| Var 6 (CNGKASC5-1A-M) | 277.73 ± 87.3 | 8 | 507.77 ± 123.0 | 4 | 144.60 ± 25.2 | 2 | 310.03 | 7 |
| Var 8 (CNGKASC6-1-L) | 166.60 ± 76.4 | 11 | 260.33 ± 49.3 | 11 | 98.33 ± 15.3 | 10 | 175.08 | 12 |
| Var 5 (CNGKASG1-0-T) | 571.70 ± 70.1 | 3 | 664.00 ± 98.3 | 1 | 104.13 ± 60.1 | 8 | 446.61 | 1 |
| Var 1 (Yamashi) | 608.77 ± 18.9 | 2 | 577.20 ± 50.0 | 2 | 98.33 ± 15.3 | 9 | 428.10 | 2 |
| Var 10 (CNGKASE-2-0-T) | 148.10 ± 79.1 | 12 | 403.60 ± 177.2 | 7 | 133.00 ± 23.1 | 4 | 228.08 | 10 |
| Var 4 (CNGKASA2-2-L) | 210.60 ± 97.7 | 10 | 546.73 ± 185.2 | 3 | 109.93 ± 32.2 | 7 | 289.08 | 8 |
| Var 7 (CNGKASH1-1-M) | 708.30 ± 109.1 | 1 | 394.90 ± 114.1 | 8 | 92.53 ± 5.7 | 11 | 398.58 | 3 |
| Mean | 382.98 ± 36.3 | | 435.98 ± 33.7 | | 119.55 ± 8.9 | | 312.61 | |
| CV (%) | 39.790 | | 45.10 | | 36.30 | | | |
| P-value | 0.009 | | 0.365 | | 0.33 | | | |

Source: analysis of our database.

They were significant ($P=0.009$) differences among genotypes at Ngandajika INERA station. Overall, the ranking method indicated that var1, var5 and var7 were best yield and more desirable genotypes across the 3 study sites Table 2.

Table-3. Analysis of combined variance, including GXE interaction according to the main effect additive model and the AMMI model.

| Factors | Df | SC | CM | F | P | TSS (%) |
|------------------|------|----------|----------|---------|-------|---------|
| Environments (E) | 2 | 2062607 | 1031304 | 64.3959 | 0.000 | 54.04 |
| Bloc (B) | 6 | 82759 | 13793 | 0.63 | 0.705 | |
| Genotypes (G) | 11 | 762059 | 69278 | 2.1366 | 0.001 | 19.96 |
| G x E | 22 | 909016 | 41319 | 1.88 | 0.025 | 23.81 |
| Residual | 66 | 1445099 | 21895 | ----- | ----- | ----- |
| PC1 | 12 | 662711.4 | 55225.95 | 27.612 | 0.000 | 73.11 |
| PC2 | 10 | 243677.1 | 24367.71 | 12.18 | 0.000 | 26.68 |
| Total | 129 | 3816441 | | | | |
| CV (%) | 45.2 | | | | | |

Source: analysis of our database.

The combined analysis of variance revealed that the genotypes x environment interaction was significant ($P=0.001$) for the 12 genotypes considered Table 3. The environment had larger proportion of the sum of squared deviations. The environmental factor represented about 54.04% of the variation. However, only 19.96% of the variation was attributable to the effects of genotypic and 23.81% to the effects of the genotype x environment interaction Table 3.

The higher value of the sum of squared deviations for the environments indicated variability in the environments studied.

The magnitude of the variability in genotype yield attributable to the environment was of 2.70%; that of the genotype-environment interaction was of 1.19%. These results indicated that there were substantial differences in the genotypic response from an environment to another one. The variance of the sum of the squares of the interaction gaps was partitioned between the IPCA1 and IPCA2 interaction.

The main axis of IPCA1 captured 73.11% of the interaction of the sum of the squared deviations. Similarly, the second axis of IPCA2 expressed 26.68% of the interaction of the sum of the squared deviations. Biplot analysis was performed and visualized to determine differences between environments (reciprocal relationship), to identify stable and adapted genotypes to the environments considered in this study. Environmentally stable genotypes were displayed in red colour in the biplot-1 Figure 2A.

The biplot AMMI-2 model Figure 2B showed that 73.1% of variability in genotype yield was probably due to environmental characteristics. While assessing the yield stability of cowpea genotypes, it was observed that most genotypes tested were scattered around the axes of the biplot. The variance that was explained by axis-1 (PC1) was of about 73.11% while that relative to axis 2 (PC2) was of 26.88%. Hence the total contribution of the axes (PC1 + PC2 = 98.99%) and the interactions due to the axes only were significant (P <0.001). In fact the average yield of all genotypes was of about 312.6361 kg / ha.

The biplots indicate that environments (sites) were not stable. This means that the environments had great variability Figure 2A and Figure 2B. Across the two biplots, varieties with red color (Var2, Var3 and Var 9) were the most stable because they were closer to zero points of axes. These 3 varieties were less affected by G x E interaction. Therefore; these three varieties are likely to yield better in environment with different characteristics.

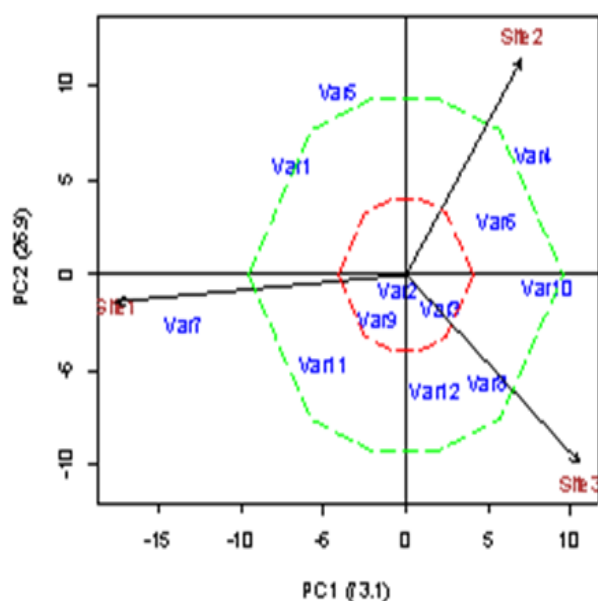


Figure-2A. Biplot for the additive effect against PC1 for the 12 cowpea genotypes in the 3 sites determined by AMMI model. Source: analysis of our database.

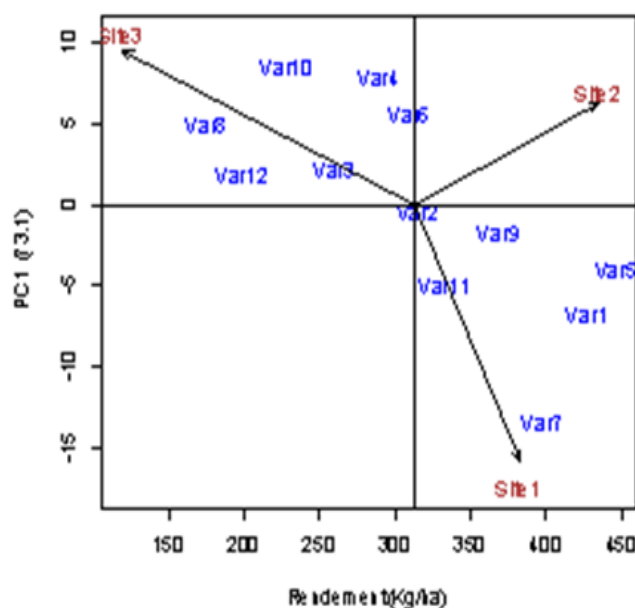


Figure-2B. Interactive effects of principal axes (PC1 against PC2) for the 12 cowpea genotypes in the 3 sites. Source: analysis of our database.

3.2. Stability Value as Determined by Ammi Model

The stability value of AMMI (ASV), provide a quantitative value of stability to enable genotype classification (ranking) with references to their levels of stability in various environments Table 4.

Table-4. Stability Value (ASV) according to AMMI Model.

| Varieties (genotypes) | ASV | YSI | rASV | rYSI | Yield (Kg/ha) |
|-------------------------|-------|-----|------|------|---------------|
| Var 2 (Mujilanga) | 1.00 | 7 | 1 | 6 | 315.34 |
| Var 9 (CNGKASC2-1-1) | 3.56 | 6 | 2 | 4 | 369.47 |
| Var 3 (CNGKASA7-2-M) | 4.08 | 12 | 3 | 9 | 260.46 |
| Var 12 (CNGKASB5-2-0-T) | 6.84 | 15 | 4 | 11 | 198.35 |
| Var 11Diamant | 9.47 | 10 | 5 | 5 | 332.22 |
| Var 6 (CNGKASC5-1A-M) | 9.62 | 13 | 6 | 7 | 310.03 |
| Var 8 (CNGKASC6-1-L) | 10.05 | 19 | 7 | 12 | 175.08 |
| Var 5 (CNGKASG1-0-T) | 11.71 | 9 | 8 | 1 | 446.61 |
| Var 1(Yamashi) | 12.66 | 11 | 9 | 2 | 428.10 |
| Var 10 (CNGKASE-2-0-T) | 14.22 | 20 | 10 | 10 | 228.08 |
| Var 4 (CNGKASA2-2-L) | 14.45 | 19 | 11 | 8 | 289.08 |
| Var7 (CNGKASH1-1-M) | 22.21 | 15 | 12 | 3 | 398.58 |

Source: analysis of our database.

The ASV measurement was used to break down the interaction effect. ASV is the distance of a variety from the zero point of the scatter plot (IPCA1 vs. IPCA2). Although the PCA1 score contributed more to the sum of squares for the G x E interaction, it may be weighed to the proportional difference between a large number of PCA1 and PCA2, in order to derive the relative contribution of PCA 1 and PCA 2 to the sum total of squares of interaction.

Using AMMI stability (ASV) model, genotype ranking in the 3 environments studied, revealed V2, V3 and V9 genotypes as the most stable genotypes compared to the rest. The analysis indicated that the remaining genotypes (V2, V4, V5, V6, V7, V8, V10, V11 and V12) were unstable genotypes although some of them yielded better in some environments [Table 3](#).

According to AMMI model, the performance stability index (YSI) revealed that the genotype V9 should be considered as a desirable genotype since it showed higher yield stability.

4. DISCUSSION

The choice of an improved genotype to be cultivated in various agro-environments with general stability and genotypic adaptation in the context of climate smart genotype has always been considered as an important issue by researchers. ANOVA and AMMI model revealed genotypes that V2, V3 and V9 genotypes were identified as the most stable across to different environments but V9 as a desirable variety.

Findings from this study indicated that the 12 genotypes had great yield variability since their yield differed from one location to the other. In addition, statistical analyses revealed that variability in genotype yield was due to environmental characteristics (attributes).

Genotypes tested responded differently to varying environmental conditions of the study sites. Environmental characteristics were diverse; they were key drivers of variability in yield. Environmental factors are classically used by breeders to select genotypes for their adaptability to various environments ([Zobel *et al.*, 1988](#); [Akter *et al.*, 2015](#)). It is important that new varieties to be released for dissemination in rural areas are selected against diverse environmental attributes. In this study, it was observed that some genotypes were associated with low yield (228 kg / ha for the variety V10) whereas others were associated with higher yields (446.61 Kg/ha for V5) as previously reported in other areas of Lomami province ([Mukendi *et al.*, 2014](#); [Mukendi *et al.*, 2017](#)).

The genotype effect is important on the yield of cowpea grain. The cropping environments of Yamba was associated with higher yields than that of INERA and Mpiana, probably because of prevailing favorable environmental conditions at both sites (high soil fertility level, good microclimate,...) as previously described ([Muyayabantu *et al.*, 2012](#)). It obviously reported that cowpea crop responds favorably to better environments.

As reported by other authors ([Bose *et al.*, 2014](#); [Vaijayanthi *et al.*, 2017](#)) genotype-environment interaction technique, was found to be useful in the identification of stable genotypes. Analysis of the G x E interaction reduces errors in the breeding ([Purchase *et al.*, 2000](#); [Mukherjee *et al.*, 2013](#)) and improves accuracy in the development and selection of genetic materials ([Kadhem and Baktash, 2016](#); [Darai *et al.*, 2017](#)). Overall, yield is a quantitative trait. The expression of the genetic potential of genotypes (quality and quantity of the yield) is known to be impacted greatly by environmental characteristics such as moisture, rainfall distribution, type of the vegetation in the surrounding landscape, types of farming practices adopted and implemented by farmers, the cropping system, the level of soil fertility ([Dolinassou *et al.*, 2016](#)).

The strong genotype x environment interaction means that it is necessary to test several locations to rank the varieties because high fluctuation in genotype yield is mostly due to environment characteristics ([Akter *et al.*, 2014](#)).

The value of AMMI stability (ASV) and yield stability index (YSI) indicates the presence of stable and desirable genotypes. Genotypes with low values of ASV and YSI are considered to be more stable and desirable genotype.

With regard to the biplots, the genotypes in the red ray and that were found to be closer to each other in the frame, tended to show similar yields across all environments (locations) as reported ([Zobel *et al.*, 1988](#)).

Genotypes V2, V3 and V9 were found to be closer to the origin of the axes; they had genotypic responses that were proportional and that were less influenced by the environment attributes. Therefore, they were predicted to display stable yield in any kind environment of Lomami Province. Scientists define stable genotypes as those that provide optimal and acceptable yield across various environments including marginal lands (Purchase *et al.*, 2000).

The genotypes (V1, V4, V5, V6, V7, V8, V10, V11 and V12) were found to be stable. They were judged as susceptible and less tolerant to high pressure due biotic and abiotic stresses. They were therefore highlighted as sensitive to environmental changes. They may require further breeding research work.

The process of setting climate-smart genotypes in the DR Congo should be based on the response of genotypes each season for better understanding the adaptation towards pressures and environmental stresses that cannot be completely reduced even when organizing the mechanism of control of the latter. The classic assumption, widely accepted by breeders, is that a genotype with high yield potential will behave well in most environments. However, this selection method did not include the concept of yield stability nor does it consider adaptation to a stress environment. These shortcomings are among the causes of slow progress in breeding and breeding on various crops in the Ngandajika area.

However, yield stability is a better indicator of genotypic performance. It is established that a high-yielding genotype in a site does not automatically indicate good performance, and low yield does not automatically indicate a genotypic underperformance. The use of the ASV and YSI indices made it possible to identify stable and desirable genotypes in the environment. Currently, ASV and YSI are among the most recommended indices for identifying high-yielding and stable genotypes in both non-stressful and stressful environments. The combination of indices should be a more useful criterion for improving cowpea genotype selection for adaptation and yield stability. The use of these indices is an attempt to improve the breeding program in the DRC compared to conventional methods of the past.

These indices can be used to guide mechanistic research aimed at understanding the basis of genotype stability and yield adaptation across different crops, since previous research focused only on genotypic performance without taking into account adaptation or stability.

5. CONCLUSION & RECOMMENDATIONS

This study was conducted to identify genotypes with greater regularity and stability of the yield on 12 cowpea genotypes, across different localities of Ngandajika territory, using AMMI model. Interestingly, AMMI model revealed genotypes with stable yields across the different environment considered in the study. The V2, V3 and V9 genotypes were identified as the most stable across the different environments retained in the study. Their yields oscillated between 260 and 369 kg / ha as compared to the overall average of 312 kg / ha. These three genotypes are recommended for dissemination in rural environments of central DR Congo.

Findings from this study indicated that there were inconsistent performances in yield of the 12 genotypes tested across the 3 environments.

The AMMI models allowed (i) to better understanding the GEI through analysis of variance, (ii) to facilitate the identification of genotypes with stable yields as well as discriminating environments via ASV and display biplot techniques and (iii) the specificity of the adaptability of genotypes to specific environments in a model taking into consideration the axes generated in the analysis.

AMMI Models provided multivariate qualitative good information that led to identification adapted and stable genotypes to various environments in central DR Congo. This finding may be used in the development of site-specific breeding genotypes as well in the mounting of selection strategies to find out stable genotypes of legume crops for the different provinces of DR Congo and across all semi-arid zones of Sub-Sahara Africa. It was therefore recommended that AMMI model tools be used in assessment of breeding program trials for some crops in DR Congo.

In addition, the development of climate-smart genotype in selection and breeding program in the DR Congo should seek to better understand the mechanisms involved in plant phenology: tolerance / resistance to high temperatures and soil infertility, as well as tolerance to stress due to drought. Developing climate smart genotypes is essential for further improvement of agricultural productivity and food security in central DR Congo. New approaches and breeding strategies should be targeted by scientists.

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REFERENCES

- Akter, A., M.J. Hasan, M. Kulsum, M. Rahman, A. Paul, L. Lipi and S. Akter, 2015. Genotype x environment interaction and yield stability analysis in hybrid rice (*Oryza Sativa* L.) by AMMI biplot. *Bangladesh Rice Journal*, 19(2): 83-90. Available at: <https://doi.org/10.3329/brj.v19i2.28168>.
- Akter, A., H. Jamil, K. Umma, M. Islam, K. Hossain and R. Mamunur, 2014. AMMI biplot analysis for stability of grain yield in hybrid rice (*Oryza sativa* L.). *J. Rice Res*, 2(2): 126. Available at: 10.4172/jrr.1000126.
- Bose, L.K., N.N. Jambhuulkar and O.N. Singh, 2014. Additive main effects and multiplicative interaction (Ammi) analysis of grain yield stability in early duration rice. *Journal of Animal and Plant Sciences*, 24(6): 1885-1897.
- Bose, L.K., N.N. Jambhuulkar and O.N. Singh, 2014. Additive main effects and multiplicative interaction (ammi) analysis of grain yield stability in early duration rice. *J. Anim. Plant Sciences*, 24(6): 1885-1897.
- Darai, R., A. Sarker, R.P. Sah, K. Pokhrel and R. Chaudhary, 2017. AMMI biplot analysis for genotype x environment interaction on yield trait of high fe content lentil genotypes in Terai and mid-hill environment of Nepal. *Annals of Agricultural & Crop Sciences*, 2(1): 1028-1032. Available at: <https://doi.org/10.26420/annagricropsci.2017.1026>.
- Das, S., R. Misra, M. Pattnaik and S. Sinha, 2011. Integrated analysis for genotypic adaptation in rice. *African Crop Science Journal*, 19(1): 15-28. Available at: <https://doi.org/10.4314/acsj.v19i1.68657>.
- Ding, M., B. Tier, W. Yan, H.X. Wu, M.B. Powell and T.A. McRae, 2008. Application of GGE biplot analysis to evaluate genotype (G), environment (E), and G x E interaction on pinus radiata: A case study. *New Zealand Journal of Forestry Science*, 38(1): 132-142.
- Dolinassou, S., T. Noubissié, A. Djirantal and Y. Njintang, 2016. Genotype x environment interaction and kernel yield-stability of groundnut (*Arachis hypogaea* L.) in Northern Cameroon. *Journal of Applied Biology & Biotechnology*, 4(01): 001-007.
- Dwivedi, S., K. Sahrawat, H. Upadhyaya, A. Mengoni, M. Galardini, M. Bazzicalupo, E. Biondi, M. Hungria, G. Kaschuk and M. Blair, 2015. Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. *Advances in Agronomy*, 129: 1-116. Available at: <https://doi.org/10.1016/bs.agron.2014.09.001>.
- INS, 2015. Annual statistics of DR Congo. Ministry of plan and modernity revolution. National Institute of Statistics. pp: 560.
- Kadhem, F. and F. Baktash, 2016. AMMI analysis of adaptability and yield stability of promising lines of bread wheat (*Triticum aestivum* L.). *The Iraqi Journal of Agricultural Science*, 47(7-special issue): 35-43.
- Kadima, M., 2006. Profile of legume sub-sector in DR Congo. FAO. Project GCP/DRC/031/BEL. pp: 53.
- Karimzadeh, R., M. Mohammadi, N. Sabaghnia, M. Shefazadeh, T. Hosseinpour and M. Armion, 2013. Exploring of genotype by environment interaction by non-parametric stability procedures. *Natura Montenegrina*, 12(1): 181-204.
- Kilic, H., M. Akcura and H. Aktas, 2010. Assessment of parametric and non-parametric methods for selecting stable and adapted durum wheat genotypes in multi-environments. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(3): 271-279.

- Lydia, H., 2016. Breeding cowpea (*Vigna unguiculata* (L.) Walp) for improved yield and related traits using gamma irradiation. Ph.D. Dissertation, ACCI, University of Kxazulu-Natal, Republic of South Africa. pp: 162.
- Mukendi, T.R., T.B. Mutamba, M.D. Kabongo, B.L. Longanza and T.M. Munyuli, 2017. Degraded soil improvement by supplementation of organic and inorganic manures and yield evaluation of maize (*Zea Mays* L.) in Lomami Province, DR Congo. *International Journal of Biological and Chemical Sciences*, 11(2): 816-827.
- Mukendi, T.R., V. Ndayinsenga, M.B. Mutamba, M.D. Kabongo, K.J. Ngoie, L.L. Baboy and T.M. Munyuli, 2017. Agronomic Evaluation of 12 cultivars of cowpea (*Vigna unguiculata* (L.) Walp) in Kabinda zone, Lomami province in DR CONGO. *International Journal of Current Research*, 9(8): 55492 – 55496.
- Mukendi, T.R., P. Tshilenge, C. Kabwe and T.M.B. Munyuli, 2014. Field assessment of the efficiency of medicinal plants against a cowpea pest (*Ootheca mutabilis* Sahlb. Chrysomelidae) in DR Congo. *Lebanese Science Journal*, 15(1): 51-72.
- Mukherjee, A., N. Mohapatra, L. Bose, N. Jambhulkar and P. Nayak, 2013. Additive main effects and multiplicative interaction (AMMI) analysis of GxE interactions in rice-blast pathosystem to identify stable resistant genotypes. *African Journal of Agricultural Research*, 8(44): 5492-5507.
- Muyayabantu, G., K. Nkongolo and B. Kadiata, 2012. Analysis of soil nutrient dynamic and residual effect following organic and inorganic fertilizations of an oxisol. *Peer Review only Journal of Plant Nutrition*, 35(1): 1-29.
- Naroui, R., M.A. Kadir, M. Rafii, H. Jaafar, M. Naghavi and F. Ahmadi, 2013. Genotype environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. *Australian Journal of Crop Science*, 7(7): 956-961.
- Noerwijati, K., T. Nasrullah, D. Prajitno and A. Nindita, 2017. Mixed model of additive main effects and multiplicative interaction for stability analysis of Cassava. *B. Life and Environmental Sciences*, 54(3): 183-190.
- Nteranya, S. and D. Bergvinson, 2015. Oilseeds and cowpeas. In *Feeding Africa, an action plan for African agricultural transformation*. October 21 – 23, Dakar, Senegal. pp: 30.
- Odehale, J., C. Ataga, C. Agho, G. Odiowaya, M. Okoye and E. Okolo, 2013. Genotype evaluation of coconut (*Cocos Nucifera* L.) and mega environment investigation based on additive main effects and multiplicative interaction (AMMI) analysis. *Research Journal of Agricultural and Environmental Management*, 2(1): 001-010.
- Owusu, E.Y., I.K. Amegbor, K. Darkwa, R. Oteng-Frimpong and E.K. Sie, 2018. Gene action and combining ability studies for yield and its related traits in cowpea (*vigna unguiculata*). *Cogne: Food & Agriculture*.
- Patel, B.D., A.M. Mehta, S.G. Patel, S. Takle, S.K. Prajapati and S.K. Patel, 2015. Genotype x environment interaction studies in promising early genotypes of rice. *Electronic Journal of Plant Breeding*, 6(2): 382- 388.
- Purchase, J., H. Hatting and C. Van Deventer, 2000. Genotype× environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. *South African Journal of Plant and Soil*, 17(3): 101-107.
- Rodrigues, P., D. Pereira and J. Mexia, 2011. A comparison between joint regression analysis and the additive main and multiplicative interaction model: The robustness with increasing amounts of missing data. *Scientia Agricola*, 68(6): 679-686. Available at: <https://doi.org/10.1590/s0103-90162011000600012>.
- Rojas-Downing, M.M., A.P. Nejadhashemi, T. Harrigan and S. Woznicki, 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management*, 16(C): 145-163. Available at: 10.1016/j.crm.2017.02.001.
- Sabaghnia, N., R. Karimizadeh and M. Mohammadi, 2013. Using additive main effect and multiplicative interaction model for exploration of yield stability in some lentil (*Lens Culinaris Medika*) genotypes. *Plant Breeding and Seed Science*, 67: 45-59. Available at: <https://doi.org/10.2478/v10129-011-0069-z>.
- Sadeghi, S., H. Samizadeh, E. Amiri and M. Ashouri, 2011. Additive main effects and multiplicative interactions (AMMI) analysis of dry leaf yield in tobacco hybrids across environments. *African Journal of Biotechnology*, 10(21): 4358-4364.
- Simion, T., W. Mohammed and B. Amsalu, 2018. Genotype by environment interaction and stability analysis of cowpea [*Vigna unguiculata* (L.) Walp] genotypes for yield in Ethiopia. *Journal of Plant Breeding and Crop Science*, 10(9): 249-257. Available at: <https://doi.org/10.5897/jpbcs2018.0753>.

- Singh., R.P., V.V. Prasad and K.R. Reddy, 2015. Climate change: Implications for stakeholders in genetic resources and seed sector. *Advance in Agronomy*, 129: 119-169.
- Vaijayanthi, P.V., S. Ramesh, A. Angadi, C.M. Chandrashekhar, M. Keerthi, P. Mahadevu and Chandrakant, 2017. Yield stability analysis of dolichos bean genotypes using AMMI model and ggl biplot. *International Journal of Agriculture Sciences*, 9(47): 4800-4805.
- Yirga, B.K., 2016. Genotype x environment interaction and yield stability of white seeded sesame (*Sesamum indicum* L.) genotypes in North Ethiopia. MSc Thesis. Dissertation. Haramaya University. pp: 83
- Zobel, R., M.J. Wright and H. Gauch, 1988. Statistical analysis of a yield trial. *Agronomy Journal*, 80(3): 388-393. Available at: <https://doi.org/10.2134/agronj1988.00021962008000030002x>.
- Zobel, R.W., 1990. A powerful statistical model for understanding genotype-by-environment interaction. In: Ed: M.S. Kang, *Genotype-By-Environment Interaction and Plant Breeding*. Baton Rouge: Louisiana State University. pp: 126-140.

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