Effect of Seed Quality and Legume Diversification on Management of Bean Common Mosaic and Bean Common Mosaic Necrosis Viruses in Western Kenya

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Abstract: Common bean (Phaseolus vulgaris L) is the main legume crop grown in Kenya, mostly by small scale farmers. Their grains are very vital in human nutrition and source of income for peasant farmers. The yield is 530 kg/ha which is lower compared to a production potential of 1400 - 2000 kg ha⁻¹ and country's production estimated at 613,902 metric tons per year. This production in Kenya has kept on declining due to poor seed quality, poor agronomic practices, abiotic and biotic factors. Biotic factors include Bean Common Mosaic Disease (BCMD), caused by Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are most wide spread with high disease incidence of upto 100% and yield loss of upto 98%. The objective of the study was; (1) to determine the effect of seed quality of common bean varieties on BCMD incidences, (2) to evaluate the effect of legume diversity intercrops on BCMV and BCMNV incidence. Trials on seed quality were laid in a randomized complete block design (RCBD) for; Rosecoco (market), Rosecoco (farm saved), Rosecoco (certified), KK8, KATX56, KK8 and KATX69 bean cultivars, randomly replicated three times. BCMD incidence and severity were calculated, recorded and leaf samples taken for serological tests. Trials on legume diversification also were laid on RCBD. Intercropped bean cultivars with other Legumes (Cowpeas and Groundnuts) and pure stands were randomly replicated three times. Incidence in both intercropped and purestand were observed and recorded. The results showed Rosecoco market had the highest incidence of 31.50%, while certified seeds had the lowest mean incidence of 24.42%. Purestands had high disease incidence (53.16%) than intercropped varieties (46.84%). Use of certified seeds and legumes intercropping with beans reduce BCMD incidences.

Keywords: BCMD, Legumes, Intercropping, Pure stand, certified seeds, Phaseolus vulgaris

1. INTRODUCTION

The common bean (*Phaseolus vulgaris L*) is the main legume crop grown in Kenya for its important value in human nutrition as it contains high protein content (Singh, 2005. Cortes et al., 2013). Its Consumption is high because it is relatively inexpensive compared to meat (Pachico, 1993). Regular consumption of common bean and other pulses is now promoted by health organizations because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (Leterme et al., 2002). In Kenya peasant farmers grow this crop also to earn an income from it by selling their grains after harvesting. The yields of 530 kg ha⁻¹ and the country production estimated at 613,902 metric tons (FAO, 2012) are lower compared to a production potential of 1400 - 2000 kg ha⁻¹ (Katungi ., 2009). Production of this crop keeps on reducing year after year due to poor seed quality, bad agronomic practices, inadequate farm inputs, declining soil fertility, unfavorable weather conditions and biotic factors. (Vasic, 2003). Virus attack reduce yield of infected plants and result into poor quality products from plant (Baboric, 2003). The bean is a short season crop, with a range of varieties maturing from 65 to 110 days (Buruchara et al., 2007). The main varieties cultivated in western Kenya include Rosecoco, Canadian wonder, KK8, KATX56, KATX69 and Pinto sugars. Rosecoco and Canadian wonder are high yielding varieties but requires heavy rains and high soil fertility to yield (Wronno *et al.*, 2001). Common bean is a warm-season crop that does not tolerate frost or long periods of exposure to near freezing temperatures at any stage of growth. The crop is not sensitive to soil type as long as it is fertile and well drained (Wortmann *et al.*, 1998

2. MATERIAL AND METHODS

Trials on effect of seed quality on BCMV and BCMNV incidence

The trials were conducted in low midland 1 (LM1). Farms were randomly selected based on (soil type, altitude, rainfall, temperature, land use, and farm typology). These farms were laid on different sites of the region to minimize environmental factors that could have influenced the results the study. Treatments were replicated 3 times and randomly applied in each replicate. On each farm, experimental plots measuring 5m x 5m were laid on randomized complete block design (RCBD). In each plot spacing was 30cm between rows and 15cm from plant to plant. Phaseolus vulgaris germplasm cultivars commonly planted by farmers in western Kenya: -Rosecoco (farmersaved), Rosecoco (market), Rosecoco (certified), KATX56 (Certified), KATX69 (Certified), KK8 (Certified)

Sampling design for data analysis

Sampling was done by removing two leaves from *P.vulgaris* plant at an interval of one metre on each row of beans.

Infected and symptomatic leaves, of each P.vulguris genotype were collected for serological analysis. Fifteen to twenty plants were sampled randomly. The sampled leaves were stored in polythene bags in a cool box then transfer to the laboratory at Masinde Muliro University of Science and Technology and stored at 4° C until it was used for analysis. The presence and absence of viral disease on common bean varieties planted was scored using a rating scale basing on (Nono-Womdim, 1996) where low incidence=1-20%, moderate incidence= 21-49% and high incidence=50-100%. Disease symptoms severity was scored on a scale of 0-3 according (Odu *et al*, 2004) of which

0 =No disease symptoms on plant,

1= Mild foliar disease symptoms,

2= Moderate foliar disease symptoms,

3= Severe distortion malformation of leaves or stem and stunting.

viral symptoms that were scored are, leaf mosaic mottling, vein banding and vein clearing, chlorotic leaves, leaf curl and crinkling, small leaves with inter-veinal yellowing, stunted growth, or a combination ofall

Legume diversification on BCMD incidences in Western Kenya

Kenya

Two intensive trials were conducted in two major bean growing agro ecological zones of western Kenya which was identified for having high disease pressure, LM1 and LM2 (Busia county), covering Madola, Bujumba, and Alupe. It was done during the long rain season of April to June, 2015 and short rain season of September to December, 2015.

Effect of Legume diversity on BCMV and BCMNV incidences

The effect of Legume diversity on BCMV and BCMNV was conducted in LM1 and LM2 in which the trials were laid on 30 farms, each measuring 10m by 10m in size, distributed in three clusters of 10 farms in each AEZ, selected based on similarities in soil type, altitude, rainfall, Temperature, Land use and farm typology. The three clusters were laid in LM1 and LM2 Butula-Busia. This comprise of Madola (cluster 1), Bujumba (cluster 2), Alupe (cluster 3). The sampling units within agro- ecological zone were farms of farmers who had been provided with seeds of different varieties of common bean- Rosecoco (market seed and farmers save), KK8 and KATX56, Groundnuts, and Cowpeas. In these farms, Global positioning system (GPS) handset (Magellan Triton[™]) was used to take co ordinates and location of the farms. Three bean cultivars, groundnut (Red Valencia) and Cowpea (K80) were intercropped with maize which is a common practice by farmers in these regions. Each bean variety, groundnut and Cowpea was planted separately within maize rows.

Spacing of legumes was 30 cm x 15 cm in the following order'

RC/KK8/KAT/GN/RC/KK8/KAT/CP/KK8/RC/KAT/GN. **KEY**: CP: Cowpea, KAT: KATX56 bean variety, RC: Rosecoco beans, GN: Ground nuts, KK8: Bean varieties. Pure stand for each variety were planted on plots of 5m by 5m in size as control experiments for BCMV and BCMNV incidences. Sampling design for data analysis was conducted as in section (3.2.1) and BCMD incidence and severity assessed and analyzed according to (to (Nono-Womdim, 1996), (0du *et al*, 2004). The viral symptoms that was scored were leaf mosaic mottling, vein banding and vein clearing chlorotic leaves, leaf curl and crinkling, small leaves with inter-veinal yellowing, stunted growth, or a combination of all.

Trials on bean varietal diversification on BCMV and BCMNV incidences

The trials were conducted in Butula-busia county on 30 farms randomly selected based on soil type, altitude, rainfall, temperature, land use, and farm typology). On each selected farm, plots measuring 10m x 10m were laid, on a Randomized complete block design (RCBD). Maize and the three bean cultivars was intercropped (Rose coco, KK8, KATX56). Each bean cultivar was separately planted in within rows of maize. Spacing for maize was 75cm x 30cm. Two bean rows were planted between two rows of maize with spacing of 30cm x 15cm in the following order. KAT/RC/KK8/KAT/KK8/RC/KAT/RC/KAT/KK8/KAT/K

K8/RC Pure stand for each bean genotype were raised in farm units 5m x 5m as control experiments for BCMD incidences with those intercropped bean genotype. The following variables were measured: -plot history, crop adjacent fields, disease levels on bean crops in the neighboring fields, inoculum levels in the soil at planting, incidences of severity of disease. The degree of BCMNV and BCMV incidences was assessed as according (Wormdim, 1996). Disease symptom severity was scored on the scale of 0-3 according to Odu *et al.*, (2004)

3. RESULTS AND CONCLUSION

BCMD incidence and severity

Viral disease incidence and severity scored were based on observed symptoms; light green or yellow leave colour, dark green mosaic pattern on leaves, puckering of leaves, distortion and rolling of leaves, mottling of leaves. Other symptoms included mottling of leaves, dwarfing of bean plant, curling and malformation of l



Fig: 3a



Figure 3a): healthy Rosecoco plant from certified seeds showing normal leaf shape and colour, taken from Alupe. B): Rosecoco bean plant raised from market seeds infected with BCMD and showing disease symptoms (Leaf puckering, dark green mosaic, curling and malformation of leaves)

Effect of seed quality on BCMV and BCMNV incidences

The viral symptoms were used to analyse disease incidence in all bean varieties obtained from agro shops, Market and farmers saved. BCMD incidences was high on Rose coco (market) with a mean of (31.500), Rose coco (farmer saved) was second with a mean of (26.786). Certified seeds showed low disease incidence: -KATX56 had a mean of (25.402), KATX69 had a mean of (25.321), Rose coco certified with a mean of (24.357).KK8 had the lowest BCMD mean incidence of (22.604).Both Rose coco (market) and Rose coco (farmer saved) indicated minimum of (17.000) and maximum of (100.000). While KATX56 showed the lowest range of disease incidence of minimum (0.000) and the maximum of (58.000). (Table 4)

Table 4: Effect of seed quality on BCMV and BCMNV incidences.

Seed variety/source	Ν	Mean	Min	Max
Rosecoco (market)	92	31.500	17	100
Rosecoco (farmer saved)	88	26.786	17	100
Rosecoco (certified)	94	24.357	0	75
KATX56-KALRO	92	25.402.	0	58
KK8-KALRO	92	22.607	0	75
KATX69-KALRO	94	25.321	0	67

Six commonly planted varieties with variant sources indicated a variation in the disease mean severity. Rosecoco (market) had the highest mean of (1.036). Rose coco (farmer saved) had a mean of (0.929). Certified seeds had lower

disease severity compared to locally sourced seeds. KATX56 had a mean (0.876), KATX69 Mean (0.842), Rose coco certified (0.786). KK8 had the lowest mean severity of (0.768). (Table 5).

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Seed variety/source	Ν	Mean	Min	Max
Rosecoco(Market)	92	1.036	0	2
Rosecoco(farmer saved)	88	0.929	0	2
Rose coco(certified)	94	0.786	0	2
KATX56-KALRO	92	0.876	0	2
KK8-KALRO	92	0.768	0	2
KATX69-KALRO	94	0.874	0	2

Table 5: Effect of seed quality BCMV and BCMNV severity

Trials on Legume diversity on BCMV and BCMNV incidences.

Three common bean varieties planted in pure stand (Rose coco, KK8 and KATX56) showed variation in BCMD mean incidences. Rose coco had the highest mean incidence (32.760), KATX56 was second with mean incidence of

(31.960) and KK8 with the lowest incidence (25.100). There was a drop in BCMD incidence when these Bean cultivars were intercropped with other legumes species (Cowpea and Groundnuts). Rosecoco (intercropped) had mean of (29.340), KATX56 (intercropped) had a mean incidence of (27.900) and KK8 (intercropped) had the lowest disease incidence

(21,900) variant BCMD incidences. (Table 6). Results indicated that if Bean varieties are intercropped with legume species, BCMD incidences reduces in its intensity.

Bean Variety	Ν	Mean	Min	Max	Std error of mean
Rose coco (pure stand)	94	32.76	00	100	2.349
KK8 (pure stand)	96	25.10	00	83	2.245
KATX56 (pure stand)	95	31.96	00	100	2.260
Rose coco (Intercropped)	94	29.34	00	93	2.159
KK8. (Intercropped)	91	21.90	00	67	2.132
KATX56 (Intercropped)	93	27.90	00	90	2.199

Table 6: BCMD incidences of legume diversification in western Kenya.

The BCMD severity mean between varieties planted in pure stand with those intercropped were compared to establish the difference in the mean. Rosecoco (pure stand) had a mean severity of (1.064) while intercropped had (1.034), KK8 pure stand had severity mean (0.969) while KK8 intercropped had Table 7. BCMD severity of Legume Diversification in western Kenya

(0.879), KATX56 pure stand had mean (1.034) while KATX56 intercropped had (0.947). These results showed a slight drop in the severity mean in all bean varieties intercropped. (Table 7).

Table 7. Devild severity of Degune Diversification in western Kenya							
Treatment	Ν	Mean	Min	Max	Std. error of mea		
coco(Pure stand)	94	1.064	0.000	3.000	0.067		

Opt. Treatment	Ν	Mean	Min	Max	Std. error of mean
Rosecoco(Pure stand)	94	1.064	0.000	3.000	0.067
KK8 (pure stand)	96	0.969	0.000	2.000	0.050
KATX56(pure stand)	95	1.032	0.000	3.000	0.062
Rose coco (intercropped)	97	1.032	0.000	3.000	0.052
KK8 (intercropped)	91	0.879	0.000	2.000	0.055
KATX56 (intercropped)	95	0.947	0.000	3.000	0.057
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The effect of Varietal diversification on BCMV and **BCMNV** incidences.

Intercropped of KK8, Rosecoco and KATX56 showed BCMD symptoms in all three varieties but with variant incidence. The level of incidence was compared with those varieties planted in pure stand as control experiment. Table 8: show the incidence in the three varieties that were intercropped. Rose coco intercropped had a mean of (27.720), while pure stand had a mean of (32.118), KK8 intercropped had BCMD incidence mean (21.596), while pure stand had mean (24.854). KATX56 intercropped had a mean of BCMD (27.559) w while pure stand had a mean of (31.747) with a minimum mean (0.000) and maximum mean (83.000)

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Treatment	Ν	Mean	Max	Min	Std error of mean		
Rosecoco (intercropped)	93	27.720	75	00	2.448		
Rosecoco Pure stand	93	32.118	100	00	2.349		
KK8 Intercropped	94	21.596	75	00	2.139		
KK8 Pure stand	96	24.854	83	00	2.345		
KATX56 Intercropped	93	27.559	75	00	2.419		
KATX56 Pure stand	95	31.747	83	00	2.260		

The effect of varietal diversification on BCMV and **BCMNV** severity.

Disease severity was based on the extent of damage in infected plants by BCMD. Intercropped bean varieties (KK8, Rosecoco, KATX56):-Showed variant BCMD severity (Table 9) in all the three varieties. The level of severity was compared with those varieties planted in pure stand as

control experiment. Rosecoco intercropped had a mean of (0.978) while pure stand had severity mean of (1.054), KK8 intercropped had BCMD severity mean of (0.840) while pure stand had mean severity (0.854), KATX56 intercropped had a mean severity of BCMD (0.978) while pure stand had severity mean of (1.000)

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Table 9: effect of var	rietal diversification on BCMD severity in	western Kenya

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	Option treatment	Ν	Mean	Max	Min	Std	error
						mean	

Rose coco (Intercropped)	93	0.978	3.000	0.000	0.062
Rose coco (Pure stand).	93	1.054	3.000	0.000	0.067
KK8 (Intercropped)	94	0.840	2.000	0.000	0.060
KK8 (Pure stand)	94	0.854	2.000	0.000	0.050
KATX56(Intercropped)	93	0.978	2.000	0.000	0.062
KATX56 (Pure stand)	93	1.000	3.000	0.000	0.062

4. DISCUSSION AND CONCLUSION

The symptoms observed in trials were mainly leaf mosaic, leaf mottling and distortion, yellowing of leaves, leave curling and malformation. Trials on seed quality showed varied disease incidence and severity. P.vulgaris germplasm from market had the highest BCMD mean incidence (31.50%) and mean severity (1.036) with variant disease symptoms. This indicates that the seeds were contaminated with a mixture of BCMD inoculum of different strains. Market seeds are obtained from different farmers which have different strain(s) of inoculum and when seeds from these farmers are mixed, inoculum strain(s) increase the degree of virulence with varied disease symptoms. This implies that the infection was seed borne since the incidence correlate with disease severity. Farmer saved beans expressed moderate incidences and severity with limited type of symptoms: - This implies that there were limited stains of BCMV inoculum in farmer saved P.vulgaris germplasm compared to market seeds. There was also a correlation between mean severity and mean incidence. This is an indication that infection was seed borne, but infected by specific strain(s) of inoculum. Certified seeds had low disease incidence and severity but with different degree of virulence on different bean genotype. This shows that certified seeds are also susceptible to BCMV but mainly transmitted by vectors rather than seeds. This is an indication that certified seeds harbor less inoculum or no inoculum strain(s), mostly infection was being transmitted by vectors (aphids) or other sources of inoculum from other hosts. Planting certified seeds is not a permanent solution to BCMD infection but only lowers its incidence. Different certified bean varieties showed a varied in BCMD incidence. It's an indication that different bean cultivars have different tolerance to BCMV and BCMNV. Some did not express viral symptoms but serologically showed the presence of BCMV, it implies that not all bean cultivars which does not show BCMD symptom are resistant to BCMV and BCMNV infection. Legume diversification lowed disease pressure and its virulence on P.vulgaris. Common bean cultivars that were intercropped with cowpeas and groundnuts had low disease mean incidence and severity on P.vulgaris than those in a pure stand. The mixtures of these legumes differ in susceptibility to BCMD stains as a result restricted spread of viruses (Wolfe, 1985), as they posse different characteristics that improved on overall disease resistance (Voss, 1988). Since BCMV and BCMNV are transmitted by aphids to P.vulgaris, they may have preferred other legume species (groundnuts and cowpeas) than bean due to difference in tastes thus lowering disease virulence on common bean. On

intercropped bean cultivars (KK8, Rosecoco and KATX56), disease incidence and severity was low than in pure stand but with varied incidences and severity from genotype to genotype. This may have been due to intercropping varieties that influenced disease incidence and epidemiology which restricted the spread of the viruses due to vectors changing their host this implies the transmission of BCMV and BCMNV inoculums reduced. There was a variation in disease incidences and severity within cultivars intercropped. Since P.vulgaris genotypes have different strain-specific resistance gene, therefore the genetic interaction between BCMV strain(s) and P.vulgaris genotypes had a relationship which make them to have a variation in virulence. That genotype with genes that were overcome by corresponding pathogenicity genes posses by the virus strain(s) became more susceptible to the virus, thus more disease incidence.

5. CONCLUSIONS

Seed quality has significant effect on BCMD incidence and severity. Seeds obtained from local market had the highest level of BCMD due to traders buying seeds from different farmers with different BCMV strain(s) resulting into BCMV mixed strains contamination. P. vulgaris germplasm from farmers also have inoculums of BCMV strains but since are from specific farms, harbor specific BCMV strain(s) may be that is the reason why their virulence is less compared to those from the Market. Certified P.vulgaris germplasm have minimum disease incidences, this was an indication that these seeds are free from inoculum. Disease symptoms observed may have been infected by disease vectors, infected plant residues or alternative hosts. Diversity of legumes species and bean varietal intercropping is one of the methods of BCMD management since it lower disease virulence. Common bean varieties have different tolerance levels on BCMV. Symptom development varies from genotype to genotype but serologically test positive for BCMV although they do not show symptom for the disease. This reveals that there are no resistance cultivars in Kenya or not known by farmers. BCMD infection is very high during short rains season than long rains seasons, it's vital for farmers to intensify vector (aphids) control and management of the disease during short rains seasons, by spraying with appropriate pesticide. P.vulgaris is more susceptible to BCMD attack during flowering stage than vegetative stage. To protect the crop, it's important to increase the application of plant nutrients and control of vectors at this stage by spraying with foliar feeds and pesticides to supplement nutrients required by the plant for production and for defensive mechanism against the viruses

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