International Journal of Environment, Agriculture and Biotechnology
Vol-7, Issue-6; Nov-Dec, 2022

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Peer Reviewed

Evaluation of the cross-pollination in maize (*Zea mays* L.) synthetic varieties grown in the High Guinean savannah zone conditions

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Received: 20 Nov 2022; Received in revised form: 13 Dec 2022; Accepted: 22 Dec 2022; Available online: 29 Dec 2022

Abstract**—** *In Cameroon, maize is the most widely cultivated cereal and is consumed by more than one third of the population. This study aimed to evaluate in the tropical conditions the cross-pollination rate in four recipient synthetic maize varieties by xenia phenomenon depending on distance to the pollen source and wind direction. The experimental design was triplicated split plot with each replication arranged as a 576 m^2 Latin square area. The combined analysis of variance showed a highly significant effect (p<0.001) of the gap from pollen source and wind direction on the cross-pollination rate of the recipient varieties. CMS 8704 yellow-grain variety which is the pollen donor and the white grain receiver cultivars CMS 2019, CMS 8501, CMS 9015 and Shaba had one to seven days' difference between the female flowering of the recipient variety and the start of male flowering of the donor. These synthetic varieties differed significantly for the number of leaves per plant, the 100-seeds weight, the plant height, and total kernels weight per plant, with cultivar Shaba showed the highest values. The highest cross-pollination rates were found in the first maize rows facing the donor field and the genetic pollution decreased with increasing distance from the donor source. At the same distance from source, the pollution level higher the North. The implementation of appropriate separation distance (>10 m) is recommended for reducing genetic pollution and ensuring coexistence of different genotypes in maize production field.*

Keywords**— *Cameroon, Cross-pollination, Genetic pollution, High Guinean savannah, Maize.***

I. INTRODUCTION

Maize (*Zea mays* L., 2n=20.) is the most cultivated plant in the world and the first cereal that enters in the diet before wheat (Garcia-Lara and Serna-Saldivar, 2019). It is a versatile multi-purpose crop, primarily used as a feed globally, but also is important as a food crop, especially in sub-Saharan Africa and Latin America, besides other non-food uses (Grote *et al*., 2021). The maize-distilling process has long been used by industry for the production of beverage alcohol. Maize thereby plays a diverse and dynamic role in global agri-food systems and food/nutrition security (Poole *et al*., 2021). Improved maize germplasm plays a prominent role in the advent of maize across the global agri-food system (Brouwer *et al*., 2020). Genetically modified (GM) maize is the second most important GM crop following soybean (Willet *et al.*, 2019). The world's total maize production was estimated at 1.05 million thousand tons in 2020 (Erenstein *et al*., 2022). Maize production of Cameroon was estimated at 2.200 thousand tons in 2020 (FAO, 2021). The current production level of maize in the country is declining and to meet consumption requirements, huge quantities of the commodity are imported (Mvodo Meyo and Mbey Egoh, 2020).

Maize is predominantly cross-pollinated with anemophily as the general rule. Maize is a wind-pollinating crop with about 95% cross pollination (Devos *et al*., 2007). Pollination with insects also takes place to certain extent (Klein *et al.*, 2007). The adaptation for cross-pollination are monoecious inflorescences, unisexual flowers, differences in time of maturity of the male and female inflorescence, silk receptive on entire length and abundant pollen production (Brittan, 2006). The intensification of maize production in order to reduce the food deficit in the face of galloping demographics requires an acceleration of the creation of new, better performing and better adapted varieties. Hybrid maize requires new materials for every crop to maintain its potential and proved a particularly viable and attractive business model for the seed industry (Morris *et al*., 2003). In sub-Saharan Africa, the development of improved open pollinated varieties (OPVs) and synthetic varieties were recommended to smallholder farmers for their performance, and their seed-recycling potential (Morris *et al*., 2003). Composite or synthetic seeds are the most appropriate for developing countries because they give farmers the possibility to renew the seed from their harvests in addition to their productivity. However, the stability of composite varieties depends in part on the level of genetic pollution which is the accidental transfer of genes between genotypes through inter-pollination (Tsai and Tsai, 1990).

Cross-pollination studies between adjacent maize fields have been conducted all over the world using mainly a color marker system (Messeguer *et al*., 2006; Njountie Tchiengue, 2010). The main focus of these studies was to gather information about adequate separation distances to ensure coexistence and about the dependence of cross-pollination on the distance within maize fields. Several studies have been performed to evaluate the impact of pollen drift from fields containing GM corn to neighboring non-GM cornfields (Byrne and Fromherz, 2003; Devos *et al*., 2005; Weber *et al*., 2007; Njontie Tchiengue, 2010; Viorica *et al*., 2017). The accidental gene flow is more pronounced under the conditions of peasant agriculture, where crop plots are close together and sometimes several varieties are grown in the same plot. The cultivation of maize in Cameroon is predominantly dominated by smallholder farmers who use traditional methods and face drudgery. The effect of genetic pollution on maize can have many consequences in particular considerable variation in vigor as well as seed yield and its components (Denney, 1992). Understanding pollen mediated gene flow is also important to achieve the coexistence measures for farming with and without genetically modified and conventional maize (Messeguer *et al*., 2006; Njountie Tchiengue, 2010). Cross-pollination is affected by many factors inducing distance between donor and recipient fields, wind direction, wind speed, flowering synchronization between donor and recipient plants, field topography, size and orientation of donor and recipient fields, pollen velocity, weather condition like any temperature and air humidity (Devos *et al*., 2005; Vogler *et al*., 2009). Most of pollen settles within 06 to 15 m of the donor plant (Brittan, 2006). The main purpose of the present study was to evaluate on some synthetic maize varieties grown in Dang (Adamawa-Cameroon) the pollen mediated gene flow depending on source-recipient distance and wind direction.

II. MATERIAL AND METHODS

2.1 Study area

The study was carried out from 2020 to 2021 at the University of Ngaoundéré experimental farm, at Dang (Ngaoundéré 3rd subdivision, Adamawa region, Cameroon), which is intersected by 7° 26' 16 4" North latitude and 13° 33' 34" East longitude and has 1115 m above the mean sea level. This region belongs to the Guinea High Savannah agroecological zone (Djoufack *et al.*, 2012). The climate is of the Sudano-Guinean type characterized with a humid trend, an average annual rainfall of 1480 mm distributed over the rainy season (March-October), and a dry season (November-March). The average annual temperature is 22.59°C, while the relative humidity is about 66.47%. The soil in the area is mostly ferruginous type developed on old basalt and has a brown reddish clay texture. There is an immense dependence of agriculture productivity on soil physicochemical properties (Nanganoa *et al*., 2020).

2.2 Plant material

The plant material used consisted of five composite maize cultivars adapted to the Guinea High Savannah agroecological zone, comprising a yellow grain (CMS 8704) using as pollen donor and four recipient white grain (CMS 9015; CMS 8501; CMS 2019; CMS 8806 and Shaba (Table 1). The seeds were obtained from the Institute of Agricultural Research for Development (IRAD, Garoua station, Cameroon).

Table 1. Characteristics of the tested synthetic maize varieties.

Varieties	Cycle (days)	Tasseling (days)	Days to female flowering	Seed color	Seed texture
CMS 2019 (receiver)	110 -115	58 - 60	61 – 64	White	Horned
CMS 8501 (receiver)	105 -110	55 - 58	59 – 62	White	Toothed
CMS 8704 (donor)	105 -110	57 - 59	58 – 61	Yellow	Horned
CMS 9015 (receiver)	90 – 95	55- 58	58 – 62	White	Toothed
SHABA (receiver)	110 – 130	61 - 65	64 – 69	White	Toothed

2.3 Field trials

During the growing season 2020, the sowing was done simultaneously, for the donor and for the pollen receiver's varieties. The experiment was laid out in a triplicated split plot design consisting of eight source-recipient distances (main treatment), four sub-treatments (wind directions), with each replication arranged as a 576 m^2 Latin square area (24.0 m x 24.0 m) (Fig. 1). In the experimental field the three blocks were spaced 120.0 m each other to avoid cross-fertilization. In the center of each square, CMS 8704 yellow-grain variety which is the pollen donor was sown inside an area of 16 m^2 (4 m length x 4 m broad). Recipients white varieties were sown each on an experimental unit consisting of one row of 4.0 m length, respectively at 1.5 m, 2.5 m, 4.0 m, 5.0 m, 6.5 m, 7.5 m, 9.0 m and 10.0 m from the pollen-donor source. Three seeds were sown per hill and one seedling was retained after thinning. Recipient varieties were sown in four different wind orientations (West, East, South and North). Maize plants were spaced 25.0 cm for receiver's plots and 40.0 cm for donor plots. All recommended agricultural practices were adopted throughout the field trials, except the application of pesticides. NPK (20% N, 10% P_2O_5, 10% KO_2) and urea (46% N) fertilizers were applied to the soil at 20 and 45 days after sowing respectively. Regular manual weeding was carried out during the vegetative phase and at flowering. At maturity, a total of 10 plants were randomly selected in each row of the recipient field for the evaluation on cross-pollination rate. On each selected plant, their main ear was collected, and the kernel number determined by counting separately white and yellow grains.

2.1 Characterization of maize genotypes

The characterization of the five synthetic varieties used in the study was done by randomly selecting 20 plants per genotype in each of the replications (five plants for each direction). The experimental design is a triplicated non randomized complete block design. Four characters selected among the maize descriptors were retained: the height of the plant (HP), the number of leaves per plant (NLP), the total kernel weight per plant (KWP), the seeds index or 100-seeds weight (SI) and the difference between the female flowering on recipient's varieties and the start of male flowering of the donor. The time difference between male and female flowering of a single plant is called anthesis-silking interval (ASI). In the case of cross-pollination, the difference in days between the female flowering of the recipient variety and the start of male flowering of the donor is ASI2 (Devos *et al*., 2005). The number of leaves per plant was obtained by manual counting on the sample of 20 plants per variety randomly selected during the flowering. The height of the plant was measured using a graduated decameter. The total kernel weight per plant and the 100-seed mass were determined using an electronic balance of 0.001g sensitivity (Sartorius Prodilab).

2.2 Evaluation of genetic pollution rate

The donor maize was a yellow grain (dominant trait) cultivar CMS 8704 and the recipient maize was white grain (recessive trait) cultivars CMS 2019, CMS 8501, CMS 9015 and Shaba. This would enable us to easily distinguish intra-cultivar pollinated and inter-cultivar pollinated grains through xenia phenomenon as recommended by Denney (1992). The xenia usually refers to a situation in which the genotype of the pollen donor influences the maternal tissue of the fruit so as to produce a phenotypically demonstrable effect upon the seed grains of the recipient. When the ovules of the recipient varieties were fertilized with the pollen of the yellow grain variety, the grains obtained appeared yellow, thereby displaying the xenia effect. Cross-pollination was investigated by the presence of yellow-grains on white-grain varieties at distance up to 17.5 m from the yellow-grain pollen (Vogler *et al*., 2009). By counting the grains showing xenia among the total grains per ear of a recipient, we could easily estimate the cross-pollination rate (P) from the following formula:

$$P = \frac{\text{Number of yellow grains}}{\text{Total number of grains}} \times 100$$

With P: the cross-pollination rate in percentage

2.3 Statistical analysis

Data obtained were subjected to the analysis of variance (ANOVA) using Statgraphics Plus Version 5.0 software. Differences in means performance were tested using the Least Significant Difference (LSD) or by the Student's t-test at 5% level of probability. Pearson linear correlation coefficient was used to assess the relationships between cross-pollination rate and distance from the source of pollen.

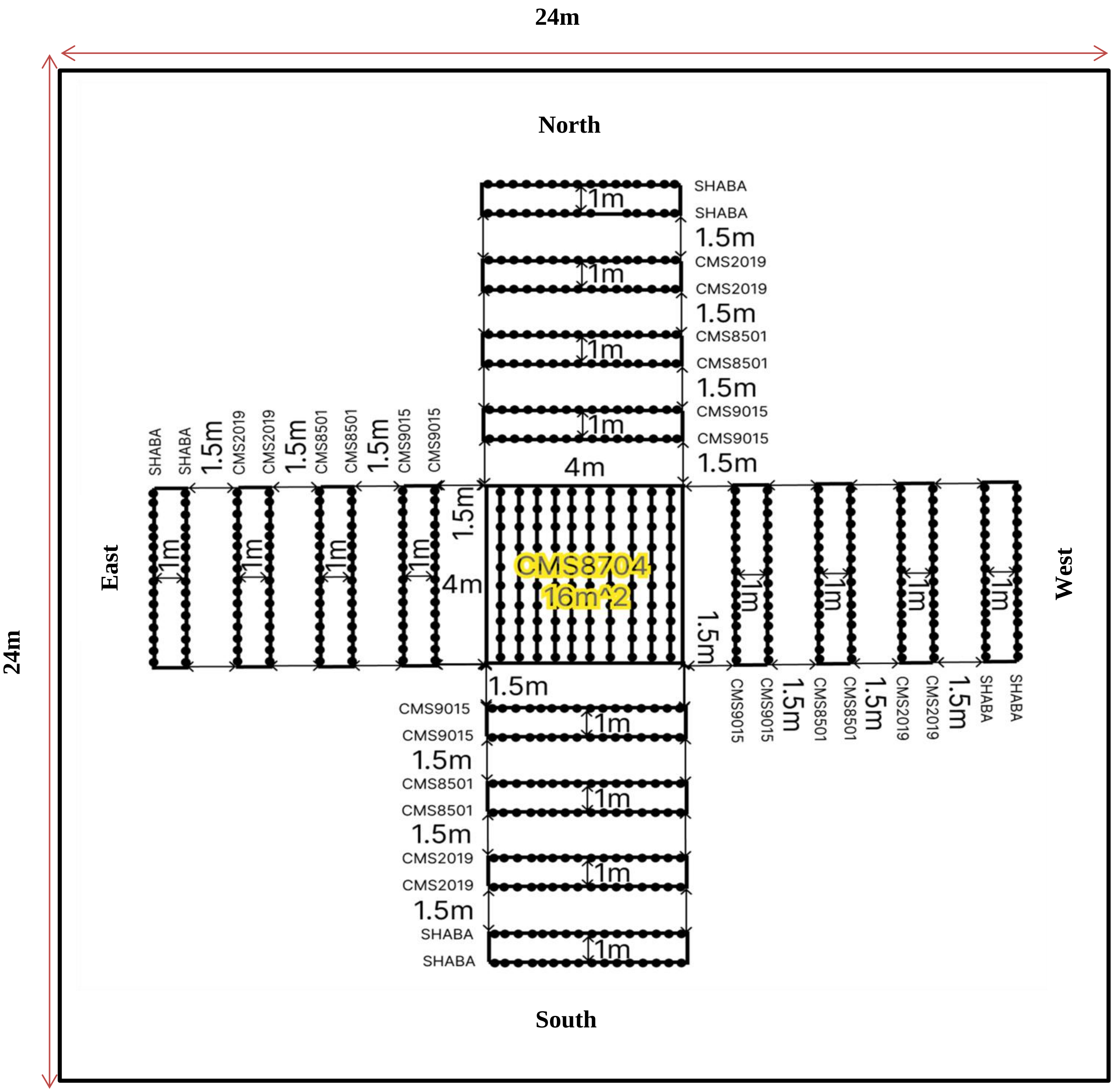

Fig. 1: Experimental layout representing pollen receiver varieties around the donor variety according to the four cardinal points (wind direction).

III. RESULTS AND DISCUSSION

3.1 Variability of tested maize varieties

The analysis of variance showed significant variability ($p<0.05$) among the five maize varieties for plant height, number of leaves per plant, seed index and the total weight of kernels per plant (Table 2). The average plant height varied from 183.0 cm (CMS 8501) to 272.21 cm (Shaba) with a mean of 207.23 cm. The height the yellow donor was slightly greater than those of the white receptor plants except Shaba. Similar results were obtained by Vogler *et al*. (2009) showing that maize height varied between 219 cm and 250 cm for improved varieties. In contrast, Viorico *et al*. (2017) in Romania observed that some improved maize varieties had tallest height.

For the number of leaves, the highest value was noted for popular Shaba cultivar (16.20 leaves) while the lowest was 14.74 leaves (CMS 8501). The stem of maize is commonly composed of 20 internodes and the leaves arise from these nodes. The number of leaves per plant noted in this study at flowering was in agreement of reports of Sangoi and Salvador (1997). The number of leaves per plant seemed to increase with the size of the plant. Plant height is a good indicator to evaluate plant growth and grain yield. The dynamic of plant height during the growing cycle could be used to access critical genetic traits, fundamental plant physiology and environmental effect. The vertical distribution of leaf is important for the analysis of photosynthesis, stress resistance and pollen propagation.

Total kernel weight per plant varied from 154.14 g (CMS 9015) to 204.18 g for Shaba with an average of 169.95 g. Drienovsky *et al.* (2019) recorded a total kernel weight per plant of 144 g to 357 g on improved varieties of maize and noted that the weight of grains could be predicted on the length of ear based on the linear equation $y = 22.5x - 156.9$. The 100-seeds weight ranged from 29.11 g for yellow grain donor variety CMS 8704 to 49.64 g for Shaba with an average of 35.72 g (Table 2). The seed index values recorded in this study were greater than values recorded by Inamullah *et al*. (2011) on maize hybrids. The variability noted for these traits could be due to the genetic diversity and environmental conditions under which the trials were conducted. There is genetic variability within cultivars for most of the agro-morphological traits. The creation and maintenance of growth and developmental homogeneity within maize population is essential.

The synchronization between of the start of male flowering of the donor variety CMS8704 and female flowering of the four white grain recipients ranged from one to seven days (Table 2). As the synchronization of yellow male flowering and white female flowering was much closer than the flowering of white male and female plants except for Shaba, cross-pollination could be expected to be unusually high (Aylor *et al*., 2003) However, rates of cross-pollination were in the expected range (Ma *et al*., 2004; Messeguer *et al*., 2006; Bannert and Stamp, 2007; Della Porta *et al*., 2008). The ASI2 depends on the genotype and environmental factors like water deficit, nutrient light and temperature (Devos *et al*., 2005). The difference in sowing dates may influence the flowering times, and limiting cross-pollination. Synchronization between donor and receiver of pollen is very important for inter-crossing between varieties.

Table 2. Genetic variability of some characteristics of tested maize varieties

Variety	**Parameters**				
	PH (cm)	NLP	KWP (g)	SI (g)	ASI2 (days)
CMS 8704	192.88 ± 6.42^{b}	14.93 ± 0.22^{b}	174.33 ± 7.11^{b}	29.11 ± 3.36^{c}	-
CMS 8501	183.0 ± 4.02^{c}	14.74 ± 0.23^{b}	156.89 ± 2.30^{c}	35.99 ± 3.35^{b}	2.0
CMS 9015	184.33 ± 6.42^{c}	15.12 ± 0.17^{ab}	154.14 ± 4.83^{c}	31.05 ± 3.36^{bc}	1.0
CMS 2019	189.47 ± 5.35^{bc}	14.76 ± 0.48^{b}	166.27 ± 9.11^{bc}	32.81 ± 2.84^{bc}	4.0
Shaba	272.21 ± 14.43^{a}	16.20 ± 0.29^{a}	204.18 ± 6.06^{a}	49.64 ± 4.67^{a}	7.0
Means	**204.37±7.32**	**15.15±0.27**	**170.76±5.88**	**35.72±3.51**	**3.5**
LSD (5%)	**8.20**	**1.01**	**16.52**	**5.22**	

Values followed by the same letter on the line are not significantly different ($p<0.05$). PH: plant height; NLP: number of leaves per plant; KWP: total kernels weight per plant; SI (g): Seed index or 100 seeds weight; ASI2: difference in days between the female flowering of the recipient variety and the start of male flowering of the donor.

3.2 Effect of the distance from pollen source and wind direction on the cross-pollination rate of recipient maize varieties

The analysis of variance showed that the cross-pollination rate of the recipient maize varieties varied significantly (p<0.001) with the distance from the pollen source and the wind direction (Table 3). The interaction between wind direction and distance from pollen source and the blocks effects were not significant. These results tell us that allogamy rates depend on the distance from the donor pollen source and the wind direction. Raynor *et al*. (1972) and Ma *et al*. (2004) noted that environmental factors and distance influenced cross-pollination in maize. The highest rates of cross-pollination were found closest to the pollen source and at further distances from the pollen source the decrease in cross-pollination was much stronger (Table 4, Fig. 2). The average pollution rate was 38.6% at 1.5 m from the source and decreased to 5.3% at 10.0 m. Cross-pollination studies between adjacent maize fields were conducted worldwide using mainly a colored marker system. Many recent studies also noted that xenia percentage was highest at the border rows facing the donor and decreased rapidly with increasing distance from the donor field (Aylor *et al*., 2003; Ma *et al*., 2004; Viorica *et al*., 2017). According to Devos *et al.* (2005), most pollen coming from donor field was retained at the border rows of recipient field that constitute a protection band, thus the proportion of donor pollen within the recipient field will decrease. The majority of the pollen deposition took place within the first two meters of the pollen source but the possibility to find a small amount of pollen at larger distances from the source exist. These observations recorded in tropical conditions were close to those obtained in Romania on improved maize varieties by Viorica *et al*. (2017) who noted a xenia percentage of 44.9% at 1 m from pollen source and 0.33% at 20 m. However, Bannert and Stamp (2007) investigated the effectiveness of distance in preventing out-crossing in maize and showed that the rate of cross-pollination ranged from 3% to 15% at 0.8 m from the donor. There is a direct correlation between the level of cross-pollination and to distance to source (R=0.928). At larger distance from the source, Aylor *et al*. (2003) noted a cross-pollination rate of 0.1 % at 50 m. Raynor *et al*. (1972) estimated that less than 1% of maize pollen grains traveled beyond 60 m, considering that maize pollen is the largest and heaviest of the Poaceae pollinated species. Dispersal distance is affected by the height of pollen release, and the topography of the surface. From these results, it appeared that at an isolation distance of 10 m, genetic pollution rate was less than 7%. These results demonstrated that spatial isolation is an effective method to reduce outcrossing rates in maize.

Concerning the wind direction (Table 3, Fig. 3), xenia percentage was highest in north (19.27%) and west (17.01%) and decreased in south (13.78%) and east (14.26%). Ma *et al.* (2004), pointed out that the cross-pollination rate was significantly higher downwind than upwind from the pollen source. Weber *et al*. (2007) noted that the influence of wind can change between locations and years, so reliable prediction is not possible. However, wind speed and direction cannot be reliably incorporated into strategies to avoid cross-pollination. Measurements of horizontal wind speed during flowering in relation to the sedimentation rate of maize pollen showed a potential distance for horizontal pollen dispersal (Bannert and Stamp, 2007). The few cross-pollinations observed over longer distances could be due to gusty or vertical wind movements (thermal or turbulence effects). According to Hofmann *et al*. (2014), most corn pollen falls within the first five meters past the edge of the field, but the possibility of finding a small amount of pollen at greater distances from the corn plot depends on wind speed.

Cross-pollination depend on other factors. A difference in flowering between the donor and recipient can reduce the level of cross-pollination (Devos *et al*., 2005). Timing between anthesis of the pollen donor and silking of the recipient is one of the main factors affecting the pollen-mediated gene. Della Porta *et al.* (2008) observed that cross-pollination depends on flowering timing. A difference of 04 to 05 days of flowering time between the pollen source and the recipient reduces the pollen flow pear to 50%. It is clear that flowering synchronization between neighboring fields is the main factor influencing cross-pollination (Messenger *et al*., 2006). The size and the ratio between the pollen source and the receiving field also influence the level of cross-pollination. The deeper the receiving field, the lower the level of cross-pollination of the crop production (Ma *et al*., 2004). Cross-pollination is considered to be responsible for much of the gene flow in maize (Devos *et al*., 2007). Gene flow influences reproductive success and fitness of individuals, and determines the genetic structure of the population.

Table 3. Analysis of variance for cross-pollination rate within the recipient field of maize tested for eight distances from pollen-donor source and four wind directions

Source of variation	**Df**	**SS**	**MS**	**F-value**
Blocks	2	13.57	6.78	0.78^{ns}
Distance from source (D)	7	2668.32	381.18	43.86^{***}
Wind direction (Wd)	3	483.90	161.30	18.56^{***}
Interaction D × Wd	21	181.22	8.63	0.99^{ns}
Residual	62	539.04	8.69	

Df: Degree of freedom; SS: Sum of square; MS: Mean of square; ns: not significant at 5%; ***: indicates significance at 0.1%.

Table 4. Impact of distance from pollen-source and wind direction on the cross-pollination rate (%) within four recipient maize synthetic varieties

Gap from pollen source (recipient variety)	**Percentage of outcrossing (%)**				
	North	**South**	**East**	**West**	**Average for distance**
1.5 m (CMS 9015)	44.64±0.28	34.67±0.41	34.94±0.38	40.24±0.31	**38.62**±0.34^{a}
2.5 m (CMS 9015)	33.33±1.51	23.04±0.62	25.24±1.05	28.23±0.99	**27.46**±1.04^{b}
4.0 m (CMS 8501)	25.31±0.53	14.88±0.57	15.32±0.60	21.92±0.38	**19.35**±0.52^{c}
5.0 m (CMS 8501)	14.97±0.59	11.99±0.85	12.47±0.85	13.35±0.44	**13.19**±0.68^{d}
6.5 m (CMS 2019)	11.92±0.85	9.13±0.39	8.93±0.16	11.02±0.62	**10.25**±0.50^{e}
7.5m (CMS 2019)	9.11±0.41	7.15±0.56	6.63±0.65	7.95±0.55	**7.71**±0.54^{f}
9.0 m (Shaba)	8.21±0.41	5.39±0.56	5.95±0.65	7.59±0.55	**6.78**±0.28^{fg}
10.0 m (Shaba)	6.72±0.43	4.03±0.37	4.65±0.25	5.81±1.00	**5.30**±0.51^{g}
Average value for direction	**19.27**±0.61^{A}	**13.78**±0.51^{C}	**14.26**±0.50^{C}	**17.01**±0.58^{B}	**16.08**±0.55

Means with the same subscript within the same column or line do not differ significantly at 5%.

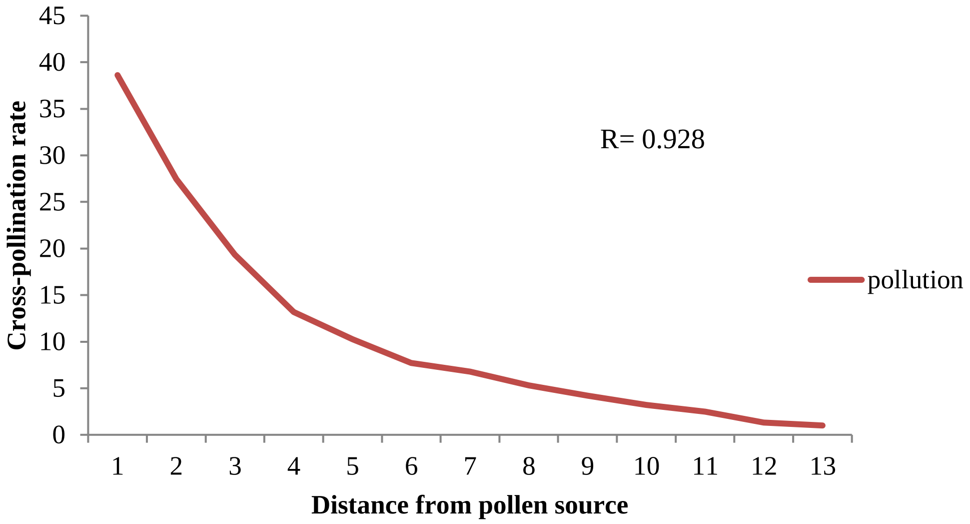

Fig. 2: Impact of gap from pollen source on the cross-pollination rate of recipient varieties.

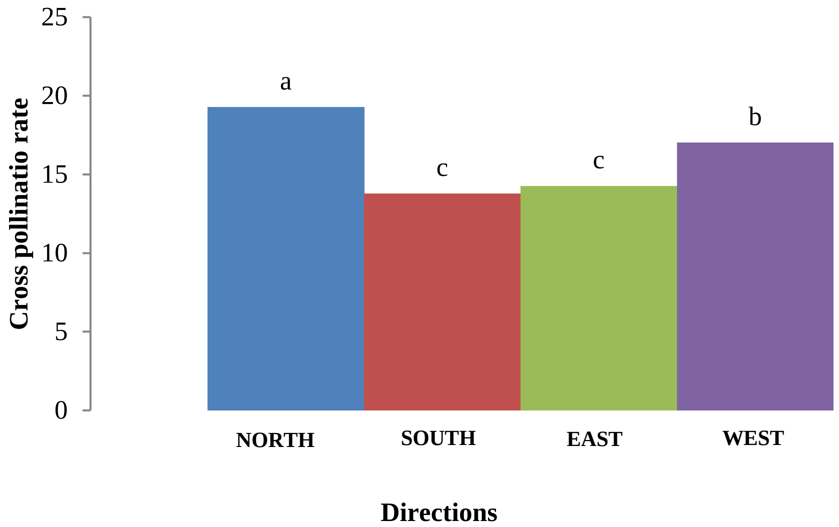

Fig. 3: Impact of wind direction on cross-pollination rate of recipient maize varieties.

IV. CONCLUSION

The main focus of this study was to gather information's about adequate separation distances to ensure coexistence and about the dependence of cross-pollination on the distance within the maize field. Results obtained showed the level of genetic pollution is highest near the pollen source and in the northern direction. At the distance of 10 m from the pollen source, the average level of genetic pollution decreased significantly in the South direction. We can recommend the cultivation at a distance of more than 10 m between maize fields to secure the coexistence of genotypes.

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