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POLLEN LONGEVITY IN ECOLOGICALLY DIFFERENT ZONES OF WESTERN KENYA

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ABSTRACT

Maize (*Zea mays* L.) is the most important staple crop in Kenya with the small holder farming systems accounting for about 75-80% of the total production. Most of the small-scale farmers plant locally adapted landraces and there are concerns about the possible contamination of these through geneflow from novel varieties, including the transgenics. The survival of pollen after dehiscence is an important factor affecting the geneflow. Studies were conducted to investigate the duration of pollen viability in two locations in western Kenya - Eldoret and Kakamega, representing the highland tropical and moist mid-altitude/transitional zones, respectively. Pollen was collected at dehiscence and exposed as a thin layer in the open air for 0 (control), 15, 30, 60, 120 and 240 minutes. Pollen viability was assessed by measuring the seed set after pollination, scoring percentage pollen color change and percentage pollen germination. Pollen maintained viability for 120 minutes after dehiscence in Eldoret (T=23-24^{0}C; RH=45-55%; Ø=-109 to -82 MPa) and for 240 minutes in Kakamega (T=25-27^{0}C; RH=68-83%; Ø=-53 to -26 MPa). The differences in pollen longevity were attributed to the differences in atmospheric water potential between the two locations. The results suggest that the likelihood of genetic contamination of the landraces through geneflow from novel varieties is higher in the moist mid-altitude zones than in the highland tropical zones of Kenya.

Key Words: Geneflow, landraces, transgenics, *Zea mays*

RÉSUMÉ

Le maïs (*Zea mays* L.) est une culture de grande importance au Kenya où, selon les systèmes agricoles du pays, la production par des petits exploitants compte environ 75 à 80% de la production globale. Principalement, la plupart des agriculteurs plantent des variétés locales et il y a lieu de s'inquiéter de la possibilité de contamination lors de la circulation des gènes à travers les nouvelles variétés, y compris la trans-génétique parce que la survie du pollen, en tant que facteur important, stimule la circulation des gènes après son ouverture. Ces études étaient menés pour examiner la durée de la viabilité du pollen dans deux endroits, à l'Ouest du Kenya: Eldoret et Kakamega, représentant respectivement la région tropicale montagneuse et la région humide d'altitude moyenne/ zones transitionnelles. En tant pondeur de mince taille, le pollen était récolté en plein air, à l'ouverture et exposé pour 0 (contrôle), 15, 30, 120, et 240 minutes. La viabilité du pollen était évaluée en mesurant la position de la graine après le transport du pollen, le pourcentage du changement de la couleur du pollen et le pourcentage de la germination du pollen. A Eldoret, le pollen avait maintenu une viabilité pendant 120 minutes après l'ouverture (T=23-24 °C); RH=45-55%; Ø=-109-82 MPa) et 240 minutes à Kakamega (T=25-27 °C; RH=68-83%; Ø=-53-26 MPa). Les différences de la longévité du pollen étaient imputables aux différences potentielles de l'eau atmosphérique entre les deux endroits. Les résultats ont suggéré que la probabilité de la contamination génétique des variétés locales pour la circulation des nouvelles variétés est plus élevée dans les zones humides d'altitude moyenne que dans les régions tropicales montagneuses du Kenya.

Mots Clés: Circulation génétique, terres habituelles, trans-génétiques, *Zea mays*

INTRODUCTION

Maize (*Zea mays* L.) is the most important staple crop in Kenya for over 90% of the population. Maize production in Kenya takes place under both smallholder and large scale farming systems, with the smallholding farms accounting for about 75-80% of the total production (Kamidi *et al.*, 1999). While large scale farmers pre-dominate some ecological zones producing large quantities of maize for commercial purposes using hybrid seed, most of the small scale farmers plant locally adapted landraces and open pollinated varieties, which are a rich source of genetic diversity for crop improvement programmes. Further, at least 80% of the maize seed planted by these farmers is produced on-farm and saved for planting in the next season.

In Kenya, among the major constraints to maize productivity, stem borers account for losses of up to 50% in all the agro ecological zones. Average yields are below 1.4 tonnes per hectare in the moist mid-altitudes zones, with 14-20% losses attributed to stem borer infestation (Hassan *et al.*, 1998). In the highlands, the average yield is 2.6-2.9 tonnes per hectare with 10-12% losses attributed to stem borer infestation (De Groote, 2002). Since 80% of Kenyan maize is produced in the transitional and the highland zones, losses to stem borer infestation could be amounting to a significant share of the potential national production (IRMA, 2001). Introduction of transgenic maize (*Bt* maize) is one of the approaches to reduce losses of maize to stem borers in Kenya (Grisley, 1997; Hassan *et al.*, 1998). The Kenya Agricultural Research Institute (KARI), in partnership with the International Maize and Wheat Improvement Center (CIMMYT) is developing transgenic maize using *Bt* constructs, as part of the project on Insect Resistant Maize for Africa (IRMA) (Mugo *et al*., 2002).

Maize is wind pollinated with long dispersion distances, and when *Bt* maize is planted by the large-scale farmers, there are possibilities that transgenes would flow through pollen drift into adjacent fields containing landraces (Ellstrand *et al*., 1999). The extent of geneflow is influenced by a number of factors, wind direction and speed, pollen water content and viability in relation to the climatic conditions during flowering (Aylor, 2004). Pollen viability was found to be relatively insensitive to solar radiation and was affected most by loss of moisture, which depends on the energy input from the sun and vapor pressure deficit of the ambient air (Aylor, 2004). It is expected that the changes in water status and therefore the survival of the corn pollen can vary according to the prevailing atmospheric conditions in different maize production systems. Temperature and relative humidity differ in the maize growing zones of Kenya (Corbett, 1998). For instance, the highland zones which are the highest maize producers, are relatively cooler and dry with mean temperatures of 23 ^{0}C and relative humidity of 55%, while the moist mid altitude zone with medium production are relatively warmer and humid with mean temperatures of 27 ^{0}C and relative humidity of 80%. Further, depending on the efficiency of artificial selection or preference by farmers and natural selection for the *Bt* trait including that resulting from pollen competition, the toxin genes that are dominant could spread to the fields and the saved seed stores of farmers who use their own varieties, though they never purchased the genetically modified varieties. Indeed, the aerial transport of genetically modified maize pollen has been viewed as a potential hazard even to non-target species (Losey *et al.*, 1999). Unfortunately the regulatory regimes in most countries are not well developed. Issues related to genetically modified (GM) maize and biosafety regulations are yet to be fully addressed in Kenya. Formulation of sound regulatory regimes will certainly require robust data and scientifc evidence relating to several attributes.

The aim of this study was to investigate how long maize pollen can remain viable under the field conditions in two agro ecological zones in Kenya. This would provide information that will help develop guidelines to minimize genetic contamination of the locally adapted maize cultivars from transgenic maize.

MATERIALS AND METHODS

The studies were conducted at the Western University of Science and Technology, Kakamega and Moi University, Chepkoilel Campus Eldoret.

Western University of Science and Technology lies at Latitude $00^0$16' N, Longitude $34^0$45' E and an altitude of 1400- 1600 meters above sea level. The mean maximum temperature is 25 ^{0}C and relative humidity ranges between 68% and 83%. The area is within the agricultural land categorized as Lower midland (LM) 1 and Upper midland (UM) 0, 1, 2, 4. Moi University, Chepkoilel Campus is at Latitude $00^0$30' N, Longitude $35^0$15' E and an altitude of 2180 meters above sea level. The area is within the Uasin Gishu plateau, which is in the lower highlands (LH3) agro ecological zone (Jaetzold and Schmidt, 1982). The site has a mean maximum temperature of 23 ^{0}C and relative humidity ranging between 45% and 55%.

The land was leveled before being demarcated, and fertilizer was applied at the rate of 75 kg N ha^{-1} and 60 kg P ha^{-1} at Eldoret and 75 kg N ha^{-1} and 25 kg P ha^{-1} at Kakamega, following standard recommendations (KARI, 1994). Two cultivars - a local variety with yellow seeds from CIMMYT, Nairobi and an open pollinated variety (H511) from the Kenya Seed Company were used in this study. The layout was a completely randomized design with each variety sown in six plots (to receive the six pollen treatments) of four rows and replicated three times. From each plot, five plants were randomly selected from the two middle rows to receive the pollen treatment to study the seed set. The plants were detasseled to avoid self pollination and ear shoots were covered with paper bags before the emergence of silk. At the onset of dehiscence, between 10.00 and 11.00 am, pollen was collected from the plants acting as the pollen source (males), i.e. yellow and H511 varieties separately. Six pollen treatments were used to study the seeds set: control (immediately after dehiscence), 15, 30, 60, 120 and 240 minutes of exposure to ambient atmospheric conditions. The pollen was sieved to remove anthers and insects using 100µm sieve, distributed as a thin layer on a white plastic tray and allowed to dry in the open air for the prescribed time after collection from the anthers. For pollination, the paper bags enclosing the ear shoots were removed and a uniform volume of pollen was applied using a camel brush onto the ears after trimming the silks to a uniform length of 4 cm. After pollination, the ear shoots were covered again with the paper bags to prevent any pollen contamination from other sources. At crop maturity, the number of kernels formed on each ear was counted to assess the viability of pollen exposed to the atmospheric conditions for different durations. Seed set was expressed as the mean number of kernels per ear, based on the counts from the five plants.

In addition to seed set study, visual scores of the percentage pollen color were made of the pollen using Munsell® color chart for plant tissues (Anonymous, 1972) under ´25 magnification. Laboratory germination test was also done on the pollen for each time period of exposure to the atmospheric conditions. The medium for germination consisted of Noble agar (7g L^{-1}), anhydrous calcium chloride (300mg L^{-1}), sucrose (35g L^{-1}) and boric acid (100mg L^{-1}) (Walden, 1986). Small amounts of pollen were then spread evenly in a thin layer on the medium and incubated for 24 hours under normal room temperatures (25 ^{0}C) and each treatment was replicated thrice.

The atmospheric water status at the two sites was assessed by calculating the atmospheric water potential (Ø atm) using temperature and relative humidity (Nobel, 1974), as follows:

$$Ø\ atm = \frac{RT}{V} \ln \frac{(\%RH)}{100}$$

Where: Ø atm = atmospheric water potential (MPa); R = Ideal gas constant (0.0083L MPa mol$É^1$deg^{-1}); T = absolute temperature (K); V = Molar volume of water (0.018 L Mol$É^1$); RH = % Relative humidity; ln = natural Log

The effect of temperature and relative humidity on the viability of maize pollen grains as measured by seed set per ear, the percentage pollen colour change and the percentage pollen germination were tested to detect significant differences among varieties in different locations using analysis of variance (ANOVA) for the two sites and the means separated using least significant difference (LSD). Additionally, Student's t- Test was used to separate the pollen germination means for variety between the two sites.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences ($P < 0.05$) in seed set with the pollen exposed for different durations to ambient conditions. Immediate pollination soon after dehiscence (control) resulted in the highest number of kernels in both the varieties, and the number of kernels decreased with increase in the period of pollen exposure in both the varieties (Table 1). A comparison of the number of kernels for each pollen treatment also showed significant differences ($P < 0.05$) between the two locations. Thus, while there was no seed set with pollen exposed for 120 minutes at Eldoret, some seed set was observed with pollen exposed to atmospheric conditions for 240 minutes at Kakamega.

Pollen viability measured by visual color changes correlated with the observation on seed set (Table 2). Pollen grains were light yellow in color at the time of collection from the anthers as expected with viable fresh pollen grains and changed to yellow and finally to deep yellow with increase in period of exposure to the atmosphere. The percentage pollen color change from light yellow to deep yellow was higher and faster at Eldoret than at Kakamega (Fig. 1). Thus, while the percentage of deep yellow colored pollen grains increased by 20% after 15 minutes of exposure at Eldoret, similar increase was observed only after 30 minutes of pollen exposure at Kakamega. Similar studies by Luna (2001) showed that viable pollen is light yellow and becomes deeper yellow or clumps when nonviable. The change in pollen color or clumping is due to irreversible degeneration that occurs in the pollen grains (Walden *et al.*, 1986).

Pollen grains incubated on media immediately after collection showed 100% germination indicating that all the pollen was viable at the two sites at the time of release from the anthers. However, in both varieties and sites, the percentage germination of pollen grains decreased with increase in time of exposure to ambient conditions. Although the percentage germination was similar across sites for the 0 (control), 15 and 30 minutes pollen treatments,

TABLE 1. Means of number of kernels per ear after pollen exposure to the ambient temperature and relative humidity for the two varieties at the two trial sites

Period of pollen exposure (minutes	Eldoret means		Kakamega means	
	H511	Yellow	H511	Yello
0	411.67	412.33	378.67	372.67
15	338.00	337.67	275.67	276.67
30	214.67	214.00	179.67	181.00
60	93.67	94.67	87.00	86.33
120	14.67	14.67	46.00	45.67
240	0.00	0.00	14.67	15.00
Mean	178.78	178.89	163.61	162.89
LSD (P=0.05)	1.68	2.65	3.02	2.10

TABLE 2. Comparison of means for the % germination, % color change and the seed set (number of kernels)

	% pollen germination	%pollen colour change	Number of kernels
Eldoret	91.6a	88.3a	323.6a
Kakamega	96.6a	98.2a	357.7a

[1]Means having a common letter within a row are not significantly different at 5% significance level according to the Student's t-Test for two samples assuming unequal variances.

differences were significant (P < 0.05) after 60, 120 and 240 minutes of pollen exposure between the two sites. Overall, pollen longevity was shorter at Eldoret as no germination was observed after 120 minutes, in contrast to Kakamega where pollen retained some germination even after exposure to atmospheric conditions for 240 minutes. There was no significant difference (P < 0.05) in the germination percentage of pollen grains between the two varieties for the period 0 - 30 hrs of exposure, within each location (Table 3). However exposure after 60 - 240 hrs exhibited a significant difference in terms of germination percentage. Similarly, the proportion of pollen grain germination differed between the two locations after a period of 60 hrs.

The results presented for seed set, pollen colour changes and pollen germinability in maize show that the pollen viability decreased with increase in time of exposure to the atmosphere before pollination. Thus, they are consistent with the previous observations which showed maize pollen is desiccation intolerant and loses viability rapidly after dehiscence (Hoekstra, 1986; Kerhoas *et al*., 1987; Barnabas, 1988; Buitink *et al*., 1996). Nevertheless, the results present here also show that pollen longevity varies between locations depending on the atmospheric conditions. Thus,

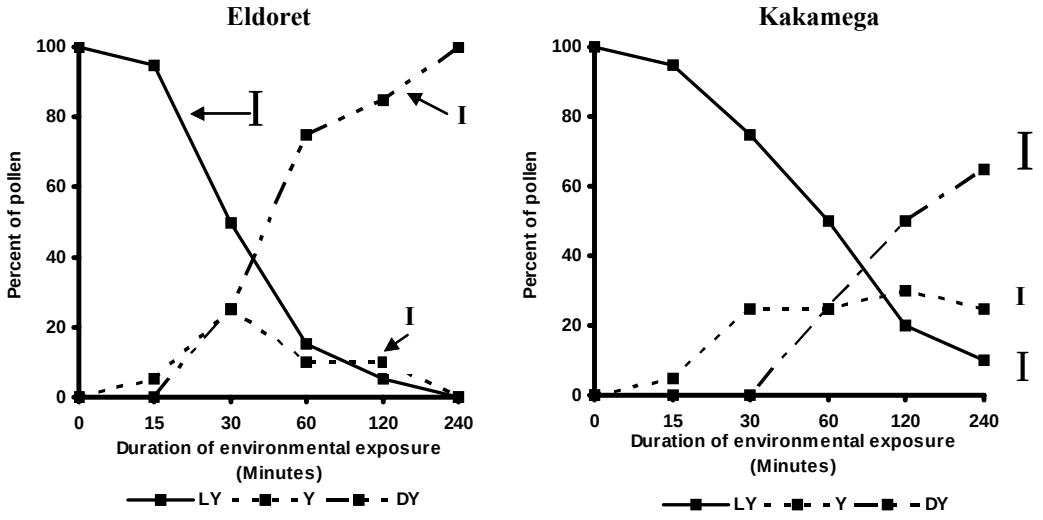

Figure 1. Changes in pollen visual appearance after being exposed to atmospheric conditions for various amounts of time. Error bars represent the LSD. (*P*d"0.05) for comparisons between the different time periods of pollen exposure. All scores were made in the field using a X25 magnification lens and the pollen color recorded using the Munsell® color chart for plant tissues. The color was recorded as Light Yellow (LY) 5Y 8/6, Yellow (Y) 5Y 8/8, Deep Yellow (DY) 5Y 8/12.

TABLE 3. Mean germination percentage in H511 and yellow maize varieties for the different period of pollen exposure in minutes at Eldoret and Kakamega

	Period of pollen exposure (hrs)					
	0	15	30	60	120	240
Eldoret	200a[1]	190a[1]	100a[1]	50a[1]	10a[1]	0a[1]
Kakamega	200a[1]	190a[1]	150a[1]	100b[1]	40b[1]	20b[1]

[1]Means having a common letter within a column are not significantly different at 5% significance level according to the Student's t- Test for two samples assuming unequal variances

pollen remained viable for a longer period at Kakamega than at Eldoret (Table 3). A study by Luna (2001) conducted in Mexico showed that, maize pollen lost viability after 120 minutes similar to that observed at Eldoret. Similar findings based on *in vitro* germination studies, revealed that the length of exposure time required for pollen germination to be reduced by 50% ranged from 60-240 min, depending on environmental conditions (Aylor, 2004).

The ability of maize pollen to remain viable during exposure in the atmosphere can potentially have an overriding effect on outcrossing distances in maize (Aylor, 2004). Pollen grains are known to maintain viability for a short period of time in hot, dry weather conditions while they remain viable for a longer period in cool and humid weather conditions. The temperature and relative humidity during pollen dehiscence at Eldoret and Kakamega ranged between 23-24 ^{0}C and 45-55% and between 25-27 ^{0}C and 68-83%, respectively. The combination of temperature and relative humidity resulted in a lower atmospheric water potential (between -109 and -82 MPa) at Eldoret than at Kakamega, (-53 and -26 MPa) during the days of pollination. The higher atmospheric water status could have contributed to the greater pollen longevity and therefore to the seed set observed in 240 minutes pollen treatment at Kakamega. Following anther dehiscence, pollen grains lose water faster to the atmosphere under low water status than under high water status and the water loss affects the viability (Barnabas, 1985; Kerhoas *et al*., 1987; Larson, 1977). It is also known that as temperature increases and relative humidity decreases, pollen grains face more arid atmospheric conditions decreasing the likelihood of cross-pollination (Baltazar and Schoper, 2002).

Maize pollen viability is affected by temperature and relative humidity in the atmosphere at the time of release from the anthers. The atmospheric conditions at Kakamega favoured maintenance of pollen viability for a longer period than at Eldoret. Kakamega, which represents the moist transitional agro ecological zone of Kenya has a wider diversity of landraces compared to Eldoret which falls within the highland tropical zone is dominated by hybrid maize and improved open pollinated varieties. There is therefore a higher risk of contamination of the maize landraces, through pollen drift from novel varieties in moist mid-altitude/moist transitional zones than in the highlands.

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