

Corn breeding for downy mildew resistant in East Timor

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Introduction

The tropical disease of downy mildew (DM), caused by the *Peronosclerospora sorghi*, is most prevalent in warm and humid regions. It has affected maize production in the Americas, South East Asia and Africa (Ullstrup et al., 1969; Exconde, 1974; Frederiksen et al., 1977; Kim et al., 2003). The DM disease has been controlled successfully by resistant cultivars. However since 2004, severe infections of DM were observed at the research stations and farmers' fields in Liquisa region of East Timor (Kim, 2004, unpublished). The same symptoms were also observed in Aileu, Betano and Lospalos in 2005, 2009 and 2010, respectively (Kim, 2005, 2009; Nabais et al., 2010). The highly infected plants exhibited symptoms of stiff, yellowish with narrow pale leaves, crazy top and no cobs formation. Only tolerant plants showed better recover with good ears and grain yield. Thines et al. (2008) reported different species of *Peronosclerospora sacchari* on sugarcane (*Saccharum* sp.) in Lospalos in 2004. We also found symptoms of Northern corn leaf blight caused by *Exserohilum turcicum* at Aileu Research Station (2006) and farmers' field in the high land farming near the Same region in 2009. At the Loes Research Station, symptoms of Southern corn leaf blight (*Bipolaris maydis*) was found in our new genotypes trials field (Nabais et al. 2010).

Incidences of DM infection are poorly understood. Infected plants with slight symptoms are considered to be susceptible. This is a common misunderstanding, that a plant must be totally free from any pathogen infection. Our breeding principle is called 'co-survival breeding' or co-environmentally friendly breeding'. Tolerant plants control DM partially and are inherited quantitatively. It is about 95% control and considered as "horizontal resistance" (Kim et al. 1977).

Breeding DMR cultivars is considered the most sustainable, economic and environmentally friendly way of tackling DM problem. Host plant tolerance breeding is an easy and economical means of controlling DM disease of maize (Ajala et al., 2003; Cardwell et al., 1997). Tolerance sources developed in Southeast Asia have been successfully deployed and utilized to control DM throughout the world. They appear to be effective against different *Peronosclerospora* species and are stable under a wide range of environmental condition (Frederiksen et al., 1970).

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⁵ Ibid.

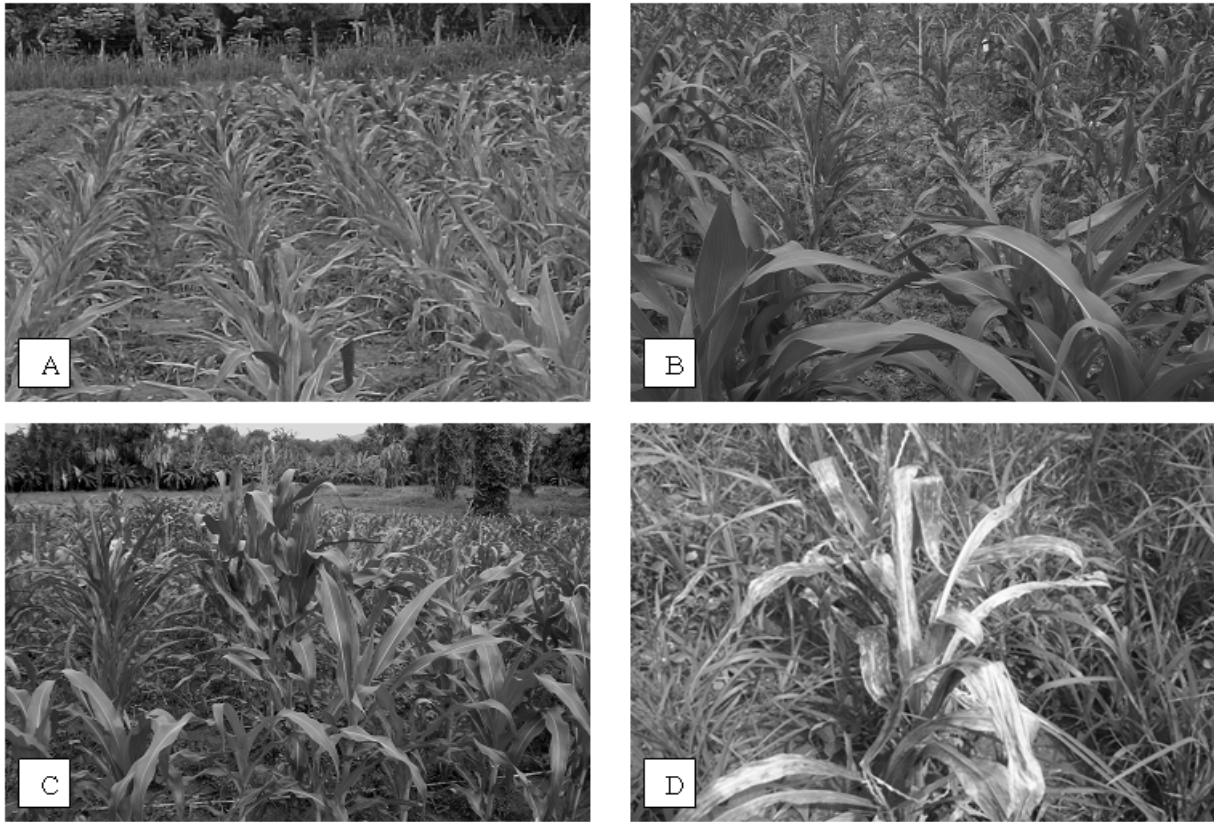


Fig.1.A. DM infection at Loes Research Station (2004). Fig.1.B. DMR genotypes tested at Loes Research Station (2008). Fig.1.C. DMR lines selected at Loes Research Station (2008). Fig.1.D. Southern corn leaf blight (*Bipolarismaydis*) infection on new cultivars tested at Loes Research Station (2008).

Materials and methods

Materials tested at Loes Research Station in 2009 and 2010

Experiment materials of DMR and non-DMR were obtained from the Gene Bank of Kyungpook National University (KNU), South Korea. The DMR source populations were DMR-ESR-Y, DMR-SR-Y (IITA), Suwan 1, Suwan 5 (Thailand) and NAI (East Timor), non-DMR from tropical and temperate regions. Genotypes were developed at KNU Farm in Kunwi and Loes Research Station (CPL) by inbreeding and selection to find DMR elite inbred lines. Materials selected were from selfing fourth (S_4) lines developed in both East Timor and Korea from 2005 to 2009. Inbred lines were developed in both East Timor and Korea to shorten the breeding cycle.

We used random mating and partial diallel crosses among the lines with similar flowering times at KNU Farm/Kunwi and CPL-East Timor during summer season in 2008 and 2009 respectively. Two sets of DMR trials compost with code KE (Korea-East Timor) 36 crosses and DM (Demonstration) 41 crosses were made. Material crosses and their parent lines were tested on December 24th, 2008 and June 17th, 2009 at the Loes Research Station in East Timor and KNU Farm in Kunwi, respectively. The parental lines of 36 genotypes were test crossed with 4 testers: Suwan 1, Suwan 5, Sele and NAI. We developed 42 crosses and tested with the 4 testers with entry code No. TL (Timor Leste) at Loes station on February 5th, 2010. Different trial materials from IITA such as EV-LSD- (Late Mature, Yellow-Grain Varieties)-MO5-2, RUVT (Regional Uniform Variety Trial) Early-MO5-4, RUVT Extra Early-M5-05, Hybrid Maize Trial

(Yellow)-For MO5-7 and Mid Altitude Inbred Lines Evaluation Trial-MO5-9 were also included. However, the data presented in this paper is only on the trials with Entry No. KE, DM and TL in East Timor.

All field trials were designed in 1 m length, 75 cm wide row with randomized complete block design (RCBD) with three replications. Seeds were planted in 25 cm between hills with 2-3 seeds per hill. Thinning was done to keep only two plants per hill. We did not apply fertilizer or pesticide in East Timor. Crops were established and managed according to the standard institute recommendations and normal farmer practice.

Investigation

The investigations were done toward the important agronomic traits, such as plant height (PH), ear height (EH), tassel length (TL), commercial value (Co.V.), DMR, ear length (EL), kernel row length (KRL), ear diameter (ED), kernel row numbers (KRN), kernel/s per row (KperR) and ear weight (EW). Data was measured with tools such as calibrated pole, vernier calipers, ruler and balance. Data analysis was done by using a Statistical Analysis Software (SAS 9.1) 2005 program.

All nurseries were exposed to a natural infection of DM. Tolerance scores were taken two and four weeks prior to flowering. DM tolerance ratings were made on determining tolerance based on 1 to 9 rating score (Ajala et al. 2003, Kim et al. 2003). Details of the DM infections were rated as follows: 1 = a few infection, highly tolerant (1-5%); 2 = a mild infection, but normal growth with a clear tolerance (6-10%); 3 = some infection, tolerance (11-20% infection); 4 = 21-40% infection, lots of infection of DM, shows some tolerance; 5 = 41-60% of infection, difficult to classify either tolerant or susceptible; 6 = 61-70% infection, lots of infections, show susceptible symptoms; 7 = 71-80% infection, considerable damage; 8 = 81-90% infection, highly significant yield loss and 9 = 91 - 100% infection, plant death.

To estimate the level of host plant tolerance, overall breeding value of the materials, here called 'commercial value' (Co.V.) of plant aspects, agronomic traits (lodging and disease and insect tolerance) and yield potential were rated based on 1 to 9 rating scale, 1= being very outstanding, 9= very poor). Kim et al. (2008) stated that the commercial value is highly correlated positively with final grain yield. The level of score from 1-9 has negative value which 1 = excellent and 9 = very poor. Commercial value (Co.V.) is the simple evaluation criterion for major traits that eliminate mean of breeding traits include tolerance to major biotic stress (disease and insect), and abiotic (lodging, drought, N deficiency) stresses, general adaptation, good agronomic traits and yield potential (Kim, 2007, unpublished). When the breeders deal with thousands of materials, they often do not have time to estimate several trait values in detail because of time and resource limitations.

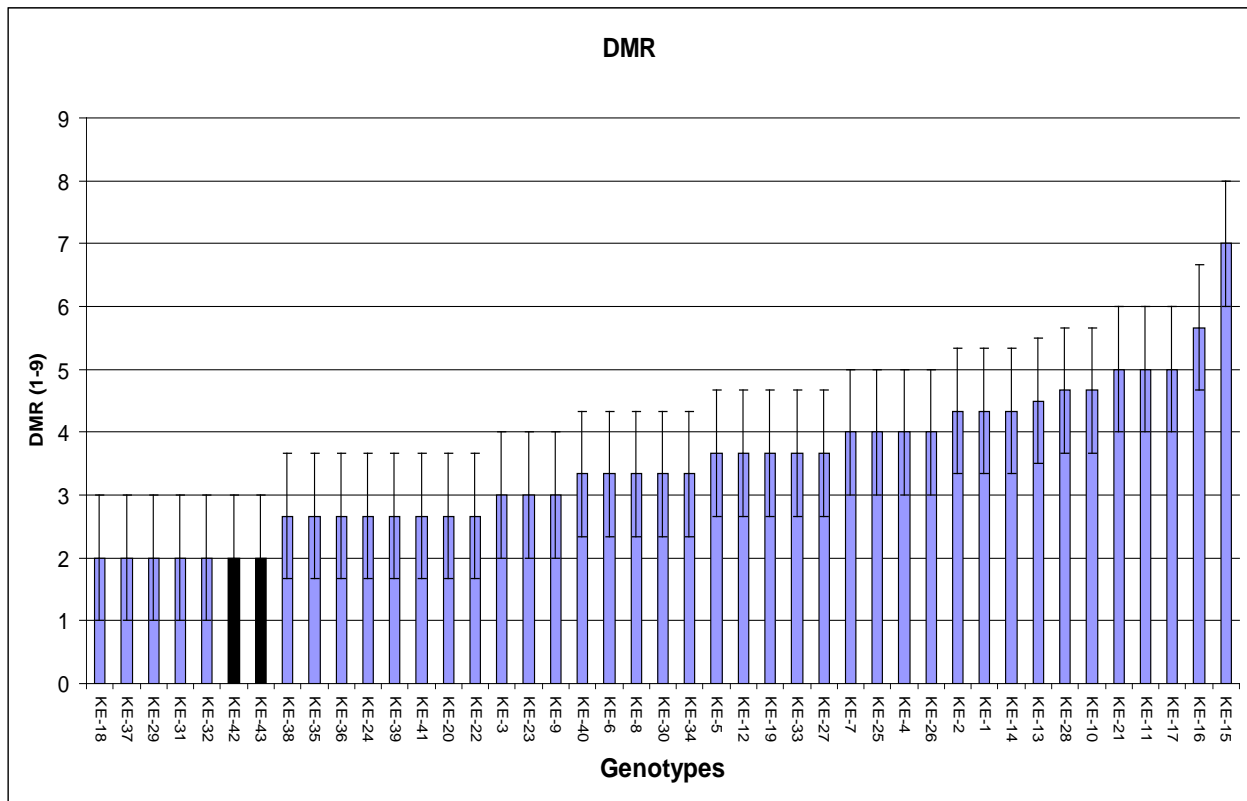
Results

Experiment 1: 41 genotypes of KE (Korea-East Timor) and 2 local checks tested for DMR

a) Downy mildew infection

Genotypes of KE exhibited different responses on DM infection from less infection (tolerant) to highly infection (susceptible). Forty three genotypes showed highly significant different ($P=0.001$: 2.0). Among of the genotypes crosses between DMR x DMR, DMR x non-DMR and non-DMR x non-DMR were easily distinguished for tolerance and susceptible. Genotypes with one of its parents from DMR sources showed tolerant with good commercial value. It revealed the importance of using DMR source populations as base lines to breed resistant lines. Genotypes of TZSTR 109 x NAI (Entry No. KE-18), (Maek x DMR) x Suwan 5 (Entry No. KE-29), (1010 x ET bulk) x TZSTR 109 (Entry No KE-31 and KE-32), Suwan 5 x TZi 25 (Entry No. 37), and two local collections exhibited tolerant to DM infection (Fig. 2). However, the result of using highly susceptible tester of TZSTR 109 (IITA) showed varied level infection of its offspring. TZi 25 was medium tolerant to DM infection.

Fig 2. DM infection on 43 genotypes tested at Loes Research Station, 2008



High coefficient of variance (CV) indicated that the variation of DM infection among the replications. Experiment with naturally infection always shows variation among the replications. The highest rates of five susceptible genotypes were Entry No. KE-15 (3503 x H5) x TZSTR 109, KE-16 (TZSTR 109 x ((P45 x JLB))), KE-17 (TZSTR 109 x Camb/Viet (*sh*)), KE-11 (2K6 ET-Local-2 x TZSTR 109, KE-21 (KV 35 x (DMR x TZSTR 109)).

b) Commercial value (Co.V.)

Commercial value (Co.V.) is an overall breeding aspect of most valuable agronomic trait such as yield, vigorous, tolerant to biotic and abiotic stresses and also lodging. The result of 43 genotypes showed highly significant different ($P=0.001: 1.2$), CV: 21.0 %, Mean: 4.2).

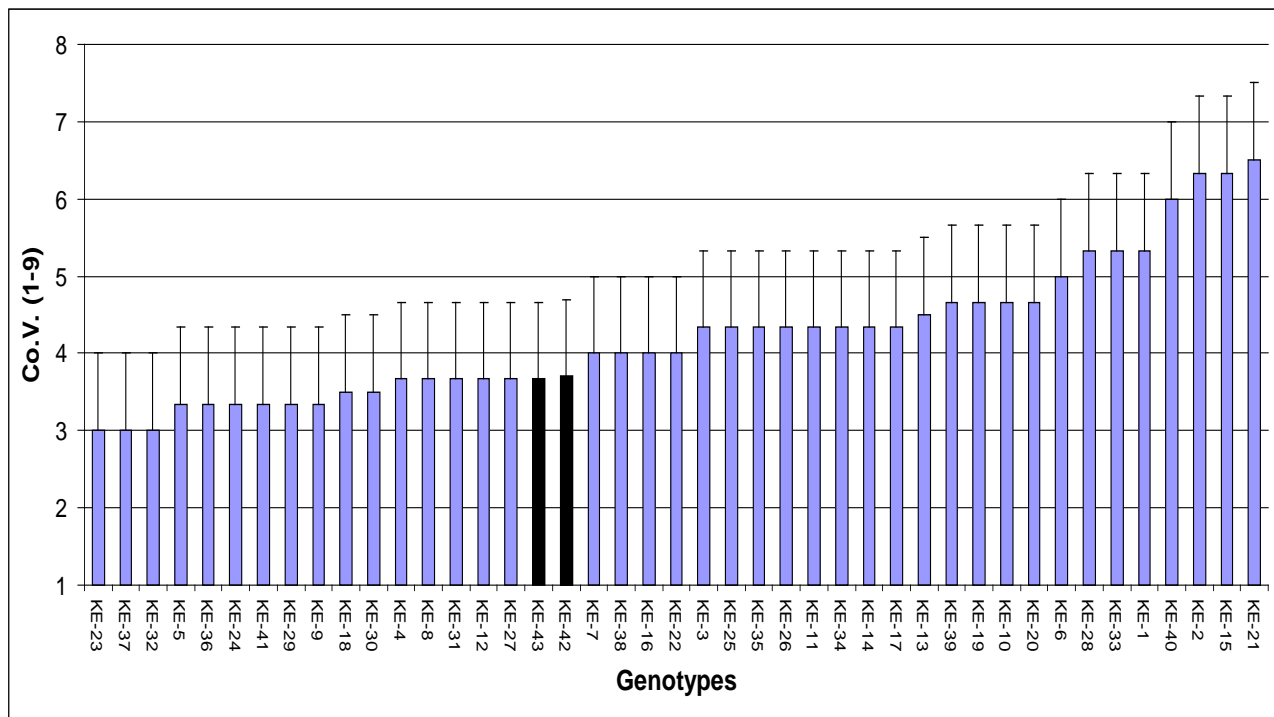


Fig 3. Commercial value of 43 genotypes tested at Loes Research Station, 2008

Entry No. KE-23 (NAI \otimes 1/3 x TZSTR 109 (small seeds)), KE-37 (Suwan 5 \otimes 2/3 x TZi 25) and KE-32 (1010 x ET bulk (2) x TZSTR 104) showed high Co.V. than other genotypes including 2 local checks (Fig 3). Entry No. KE-21 ((KV 35 x DMR) x TZSTR 109 (small seeds)), KE-15 ((3503 x H5) x TZSTR 109 (small seeds)), KE-2 (Lao-LVN-10 x TZSTR 109 (small seeds) and KE-40 (MO17 x Suwan 5) exhibited highly susceptible than other genotypes.

c) Plant height (PH)

Plant height is a quantitative trait controlled by more than one gene. It is easily affected by the environment such as plant density, nutrient, disease infection, water availability and genetic background. The result of 43 genotypes showed highly significant different ($P=0.01$: 45.3; CV :6.8 and $Mean$: 165.7). Entry No. KE-42 (Local check 1), KE-37 (Suwan 5 \otimes 2/3 x TZi 25), KE-29 ((Maek x DMR) x S5) x TZSTR 104)) and KE-24 (NAI \otimes 2/3 x TZSTR 109 (small seeds)) exhibited the highest plant height than other genotypes (Fig. 4). Five shortest plant heights were Entry No. KE-15 (3503 x H5) x TZSTR 109 (small seeds), KE-21 (KV 35 x DMR) x TZSTR 109 (small seeds), KE-10 (38(8A1) x JLB) x TZSTR 109 (small seeds), KE-19 (TZSTR 109 (small seeds) x TZi 25) and KE-33 (TZSTR 104 x TZi 25).

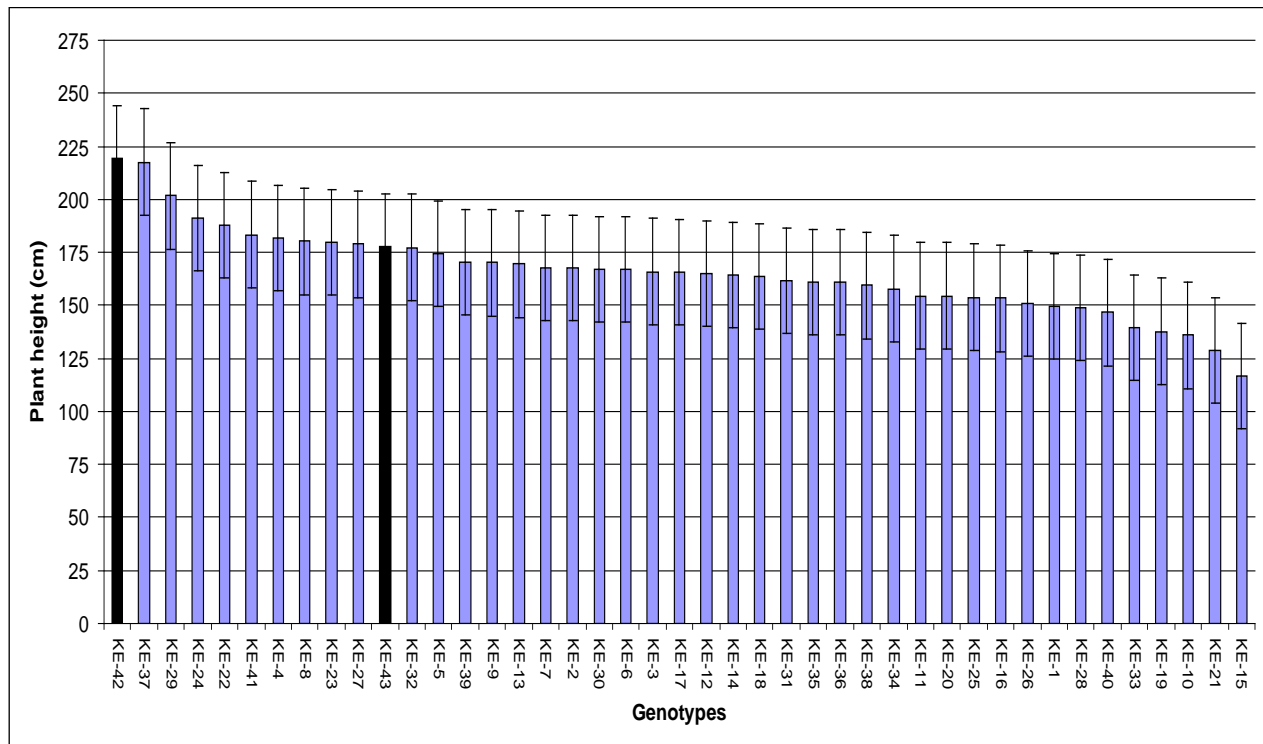


Fig 4. Plant height of 43 genotypes tested at Loes Research Station in 2008

Experiment 2: Multi-environment test of 24 genotypes of DMR and non-DMR in East Timor and Korea

a) Plant height

Twenty four genotypes were tested in two different ecological zones, East Timor and Korea. Materials were planted during the rainy season in December, 2008 in East Timor and the summer season in June, 2009 in Korea. The result showed no significant difference among the replications ($P= 0.87$), genotypes ($P= 0.09$) in each location and between genotypes versus replications.

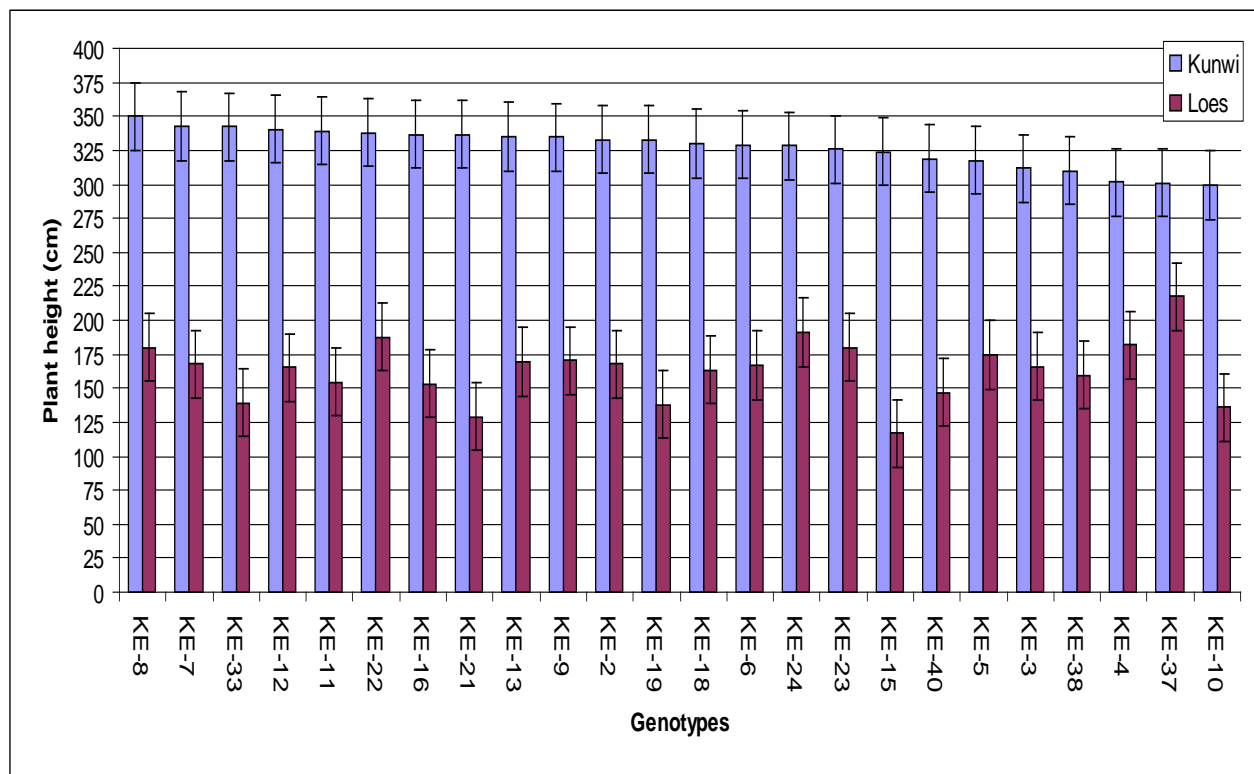


Fig 5. Environmental effect on plant height of 24 genotypes tested in East Timor and Korea

However, there was highly significant difference ($P= 0.001$) for the environment in East Timor and Korea. Genotypes showed responses on different effect of environmental change. Genotypes with Entry No. KE-37 (S5 \otimes 2/3 x TZi 25), KE-22 (NAI \otimes 1/3 x TZSTR 109 (small seeds)) and KE-24 (Camb/Viet (*sh*) x TZSTR 109 (small seeds)) (Fig. 5) exhibited the highest ($P= 0.005$) PH in East Timor were highly affected ($P= 0.001$) by the environment in Korea. Genotypes and environment were significant different ($P= 0.02$) for both places.

b) Ear height

The trait of ear height of 24 genotypes tested in East Timor and Korea showed highly significant different ($P= 0.001$). Genotypes exhibited different responses with no significant at $P= 0.84$ for replications and $P= 0.2$ for genotypes versus replications. However, genotypes showed highly significant difference at $P= 0.001$ for environmental effects and $P= 0.004$ for the interaction of genotypes and environment. Among 24 genotypes revealed significant difference at $P= 0.02$ for genotypes versus environment.

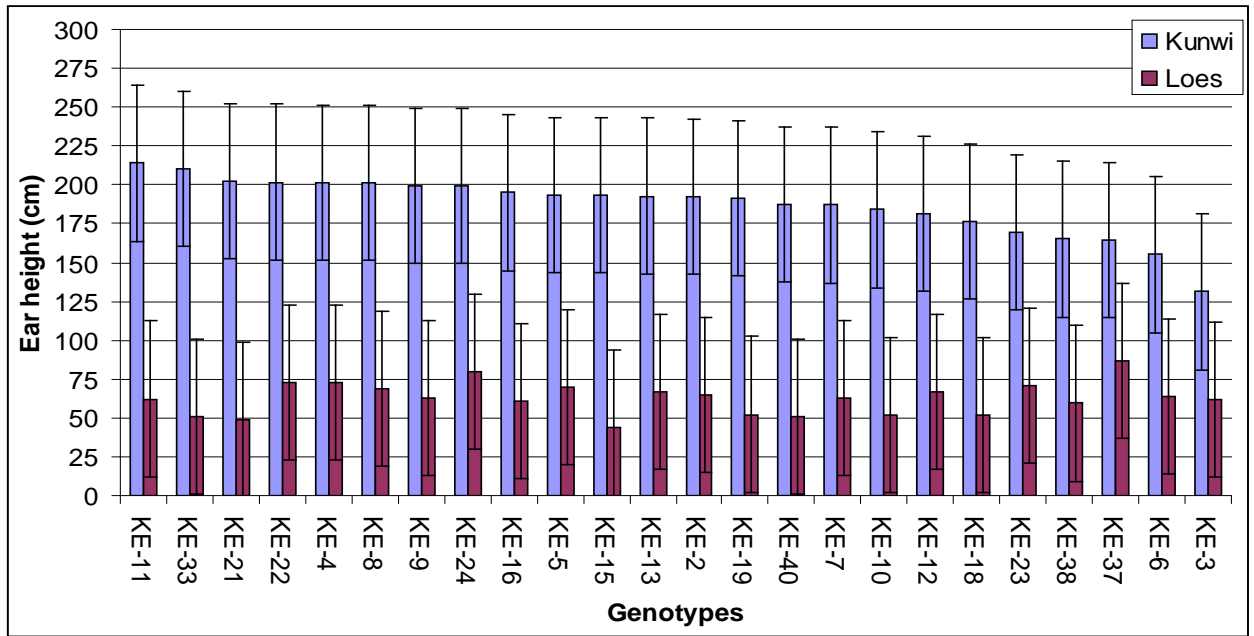


Fig 6.Environmental effect on ear height of 24 genotypes tested in East Timor and Korea

It is clearly revealed that the location of ear is highly affected by the environment. Genotypes showed different responses due to the environmental effect in East Timor and Korea. Ear height is not a fixed portion of location which ear is attached at the plant (stalk), but it maybe moved to the higher or lower nodes that depend on the environment (we did not observe). So, this trait is controlled by many genes.

c) Commercial value

The commercial value was rated from the mean value of the important agronomic traits, such as grain yield potential, disease tolerant, lodging tolerant, vigorous and high yield of dry matter. The result showed highly significant difference at $P= 0.001$. Among the replications and genotypes versus replications were no significant difference at $P= 0.73$ and $P= 0.28$, respectively (Table 2.4). Three components such as genotypes, genotypes versus environment and environment showed highly significant different ($P= 0.001$). Entry No. with highest mean for Co.V. for both East Timor and Korea environment were KE-12 (1010 x ET bulk) x TZSTR 109, KE-18 (Camb/Viet (*sh*) x TZSTR 109, Ke-24 (NAI \otimes 2/3 x TZSTR 109, KE-13 (Myanm (*sh*)/normal x TZSTR 109 (small seeds)), KE-23 (NAI \otimes 1/3 x TZSTR 109)) and KE-04 (Lao Dud Lcl black x Bisi-2) x TZSTR 109)) showed highest Co.V. than other genotypes (Fig. 7).

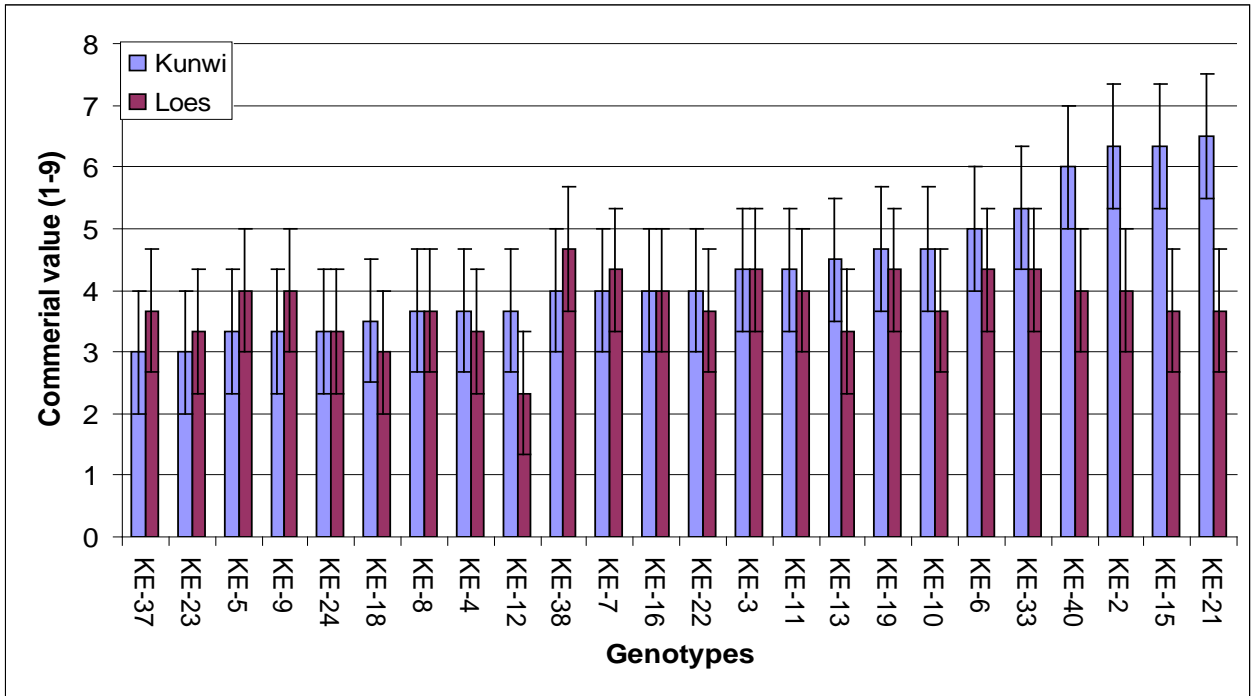


Fig 7.Environmental effect on commercial value of 24 genotypes tested in East Timor and Korea

Experiment 3: Yield test of 41 crosses and 2 checks tested at Loes Research Station on January 22nd, 2010

a) Commercial value

Forty one crosses were developed at KNU Farm during Summer in 2009. We used 4 common cultivars (Suwan 1, Suwan 5, Cele and NAI) of East Timor as testers. Inbred lines were parental lines of KE genotypes. The result showed significant ($P= 0.046$) for two highest Co.V.: TL-37 (ET-39 x Suwan 5) and TL-33 (ET-21 x Suwan 5) (Fig 2.11, Appendix 1). ET-39 and ET-21 were generated from the population of Bisi-2, DMR-ESR-Y and East Timor pop (3066). Other lines exhibited moderate were TL-04 (ET-22 x Cele), TL-14 (ET-43 x NAI), TL-28 (ET-07 x Suwan 5), TL-30 (ET-16 x Suwan 5), TL-31 (ET-20 x Suwan 5) and TL-40 (Suwan 5) (Fig. 8).

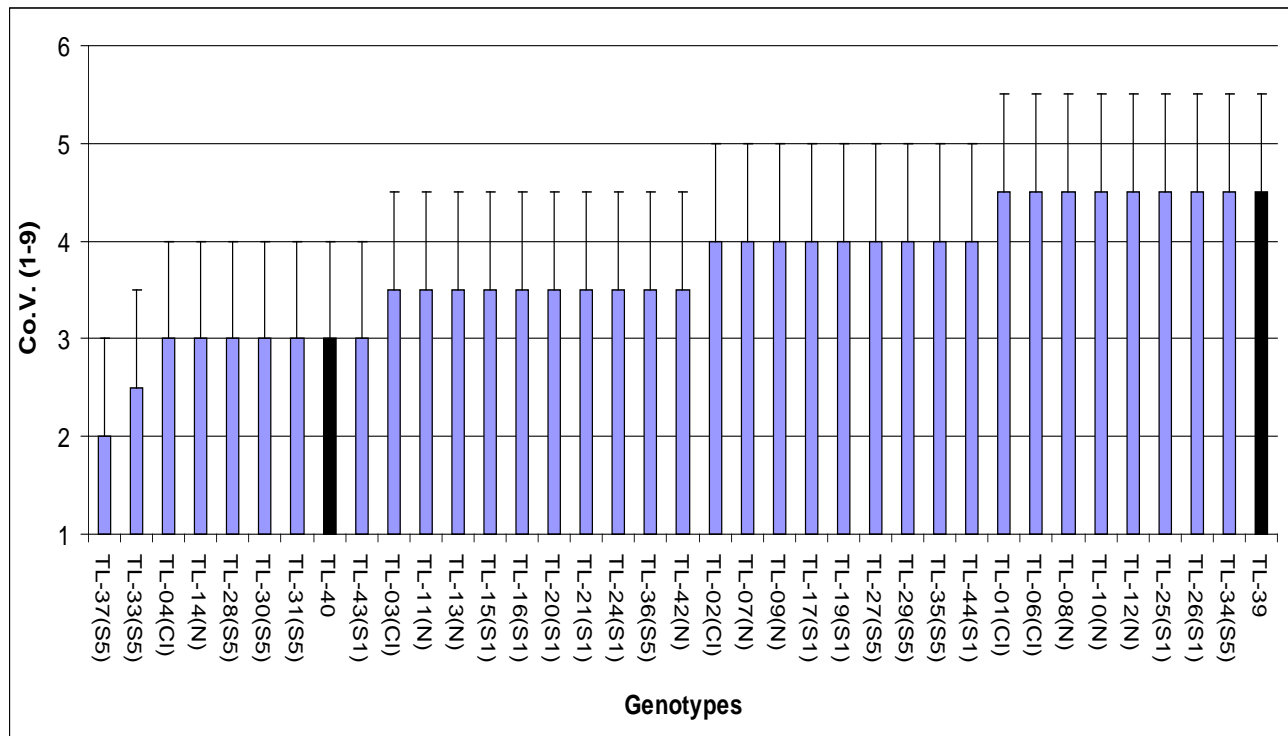


Fig 8. Testcross of 41 genotypes and 2 checks tested at Loes Research Station in East Timor

TL-37 and TL-33 were considered the highest yield potential for hybrid corn for East Timor. Inbreeding for parental lines of TL-37 and TL-33 are important to increase the homozygous of inbred lines. Further selection, evaluation and testcross are necessary to screen only desirable plants.

Discussion

Genotypes of KE exhibited different responses on DM infection from less infection (tolerant) to highly infection (susceptible). Genotypes with DMR background exhibited more tolerant with high commercial value. The result showed a correlation between DM infection and Co.V. Plants with highly infection showed highly correlation with Co.V. Plants with highly infection exhibited poor with low yield. However, different lines with non-DMR and DMR also exhibited variations of DM infection. The result showed that genotypes of TZSTR 109 x NAI (Entry No. KE-18), (Maek x DMR) x Suwan 5 (Entry No. KE-29), (1010 x ET bulk) x TZSTR 109 (Entry No KE-31 and KE-32), Suwan 5 x TZi 25 (Entry No. 37), and two local collections exhibited tolerant to DM infection. The result of using highly susceptible tester of TZSTR 109 (IITA) showed varied level infection of its offspring. Breeding for high tolerance cultivars for DM by using adapted cultivars will play a catalytic role to reduce DM spread in the country. Our experiences in DMR breeding showed that breeding high resistance lines is difficult. However, breeding for host tolerance with polygenic system with a threshold nature is possible. Resistance to DM was polygenic and quantitatively inherited (Kim, 2000; Kim et al. 2003, Singburadom and Renfro 1982).

Tropical Suwan 1, Suwan 5, NAI and Sele showed moderate performance at KNU Farm in Kunwi, South Korea although produced less yield production than new developed genotypes. Betran et al. (2003) stated that tropical corn has a broad genetic base that shows greater genetic diversity than temperate corn. The crossed lines of Mo17 and Suwan 5 with the lowest Co.V. at Loes station in East Timor but exhibited the highest yield at KNU Farm in Kunwi. Tropical materials, however, showed tolerance to the environment at KNU Farm.

The results of the experiment showed that most inbred lines crossed with Suwan 5 exhibited strong performance at Loes station in East Timor. Suwan 5, developed in Thailand, showed a high combining ability. It is considered a good tester for new inbred lines development. Inbreeding for parental lines of TL-37 and TL-33 are important to increase the homozygous of inbred lines. Further selection, evaluation and testcross are necessary to screen only desirable plants.

Conclusion

Downy mildew (DM) is an important disease in East Timor. Breeding by using DMR population (Sele, NAI, Suwan 1 and Suwan 5) is considered the most important breeding for a sustainable, economic and environmentally friendly way of tackling the DM problem. Breeding for high tolerance cultivars for DM by using adapted cultivars will play a catalytic role in reducing DM spread in the country. Breeding to get high resistance lines is difficult. However, breeding for host tolerance with polygenic system with a threshold nature is possible. Depending on the parental genetic background, the gene actions for tolerance of individual plants and means are varied. Our experiences revealed that genotypes with one of its parents from DMR sources showed tolerant with good commercial value (Co.V).

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