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RESEARCH ARTICLES

EVALUATION AND SELECTION OF MAIZE INBRED LINES FOR RESISTANCE TO FUSARIUM STALK ROT IN VIET NAM

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ABSTRACT

Identifying the resistant genotypes is one of vital strategies to control Fusarium stalk rot disease in maize. Twenty-four maize inbred lines were evaluated for resistance to stalk rot caused by *Fusarium verticillioides* at Crop Research and Development Institute (CRDI), Vietnam Nation University of Agriculture (VNUA) during the spring of 2016, and their QTL resistance were also studied at the molecular level using SSR maker with two specific primers reference from Jun-Qiang Ding et al, 2008 reported to avoid environmental effects in the JICA laboratory, VNUA, Vietnam. Disease severity was calculated in the field using a severity scale (SS) and direct estimation of stalk discoloration (SD) by artificial inoculation method. Both SS and SD results combined molecular marker was identified 18 inbred maize lines were resistant to *Fusarium* stalk rot. Disease assessments based on SS, SD were significantly correlated ($r = 0.85$; $P < 0.01$) in spring, respectively. SSR marker and SD were significantly correlated ($r = 0.85$; $P < 0.01$). Eighteen genotypes showed highly resistant response in growing seasons are D_1, D_2, D_3, D_4, D_5, D_6, D_7, D_8, D_9, D_{10}, D_{11}, D_{12}, D_{14}, D_{15}, D_{17},D_{19}, D_{20} and D_{21}, these lines can be utilized to develop the maize hybrids resistant to *Fusarium* stalk rot in Vietnam.

INTRODUCTION

Stalk rot disease of maize, caused by *Fusarium verticillioides*, is one of the most widespread and damaging diseases throughout the world, resulting in serious yield losses (De Leon C. et al, 1989; Afolabi *et al.,* 2008). *Fusarium* is a common pathogen of maize causing root, stalk and ear rot worldwide, it is more widespread in tropical and subtropical region. This is arising from the ability of most species to produce mycotoxin called fumonisins. This mycotoxin is of particular concern because it is believed of being carcinogenic (Prelusky *et al.,* 1994), linked with neural tube defects in human (Missmer *et al.,* 2006) and causes severe diseases in a variety of livestock (Morgavi and Riley, 2007). Recently years, Vietnam maize production was developed in the impressive includes the area, yield and productivity, maize grown area in 1961 are 260.2 thousand hectares increased 1,170.3 million Hectares in 2013, yield from 1.12 t/ha in 1961 gone up to is 4.43 t/ha in 2013 and production quantity from 2.92 million ton in 1961 gone up to 5.19 million ton in 2013, which were increased 1.77 times (FAOSTAT, 2014). But, Vietnam maize production is meeting challenges that are abiotic and biotic stress, among biotic stress, the stalk rot disease is impediment for production and animal feed industry. Therefore, the objective of this study was to evaluate CRDI's maize inbred lines for resistance to *F. verticillioides* stalk and ear rot under field and artificial infection and also using molecular marker to detect major QTL control the resistance. Inbred lines possessing resistance to stalk rot would be valuable breeding stock for use as parents of maize hybrids.

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MATERIALS AND METHODS

Planting material and field site: Twenty - four maize inbred lines were developed from Crop Research and Development Institute, Vietnam Nation University of Agriculture (VNUA) from local and exotic germplasm; all of the inbred lines were planted in the field to evaluate their resistance to *Fusarium* stalk rot caused by *Fusarium verticillioides* in the growing seasons, spring of 2016. The study was conducted in spring 2016 at the CRDI is located in the Red River Delta region, the experimental design was RCBD with 3 replications in a single-row plot 5m long, spaced 0.70 m between rows, 0.25 m between plants within rows.

Inoculation and disease assessment: *Fusarium verticillioides* (Sacc.) Nirenberg was harvested from susceptible lines from difference regions are Isolate 1, Isolate 2, Isolate 3 and Isolate 4. To obtain large amounts of inoculants for field inoculation, the spores were cultured on potato dextrose agar (PDA) media with subsequent subculturing on cooked maize grains at room temperature for 30 days prior to collection for inoculations at the Tropical plant disease Centre, VNUA. In each plot 8–10 uniform plants were selected and the middle part of the stalk of

each plant was inoculated. Inoculations were performed by the sponge and nail punch method (Meena Shekhar & Sangit Kumar, 2012) using a conidial suspension of 1 x 10^6 spores/ml. Disease symptoms appeared in the inoculated plants about 20-25 days after inoculation. The disease intensity and severity is recorded following 1-9 rating scale as described below: (1) Healthy or slight discolouration at the site of inoculation; (2) Up to 50% of the inoculated internode is discoloured; (3) 51-75% of the inoculated internode is discoloured; (4)76-100% of the inoculated internode is discoloured;(5) Less than 50% discolouration of the adjacent internode; (6) More than 50% discolouration of the adjacent internode; (7) Discolouration of three internodes; (8) Discolouration of four internodes; (9) Discolouration of five or more internodes and premature death of plant. The study from Jun-Qiang Ding et al, 2008 used SSR markers and indicated that two major QTL on chromosome 3 (bin 3.04) was consistent major resistant QTL with the largest effect was flanked by markers *umc1025* and *umc1742* on chromosome 3 (3.04 bin), explaining 13–22% of the phenotypic variation. The SSR markers closely flanking the major resistance QTL will facilitate marker-assisted selection (MAS) of resistance to *Fusarium* rot in maize breeding programs.

We were used SSR maker to detect major QTL in 24 maize inbred lines, SSR primers and PCR amplification include Four pairs of microsatellite primers (*Umc1742* forward*, Umc1742* reverse*, Umc1025* forward and *Umc1025* reverse) according to Jun-Qiang Ding et al. 2008were exploited to find out the genetic variations among the selected lines. DNA for genotyping was extracted from powdered leaf material of a sample of 5 plantlets of each lines and using the cetyltrimethylammonium bromide (CTAB) method (Hoisington *et al.,* 1994). PCR amplifications of the SSRs were executed in 50 µL reactions containing 20 ng of genomic DNA, 1 µL of each primer (forward and reverse), 1 µL of dNTPs, 5 µL of 10X buffer for Taq polymerase, and 2.5 U of Taq DNA polymerase; the remaining volume was completed with the addition of autoclave water. Amplification was carried out in a 1000 Touch Thermocycler programmed for a first denaturation step of 4 min at 94 °C (initial denaturation), followed by 40 cycles of 1 min at 94 °C (denaturation), 54 °C for 1 min (primer annealing), and 72 °C for 120 s. interaction effects of QTL detected for Fusarium stalk rot resistance. Gel electrophoresis and imaging, three grams of agarose was added to 0.5X TBE solutions to form 1.5% agarose gel. Ethidium bromide solution was added to the agarose gel for staining purpose. About 3 µL of the PCR products were loaded after mixing with 7 µL of gel loading dye (bromophenol blue) in a reaction volume. Testing the significance of genotype difference: Data collected was initially subjected to analyses of variance (ANOVA), $LSD_{.05}$ was performed using the IRRISTAT version 5.0. The populations were considered as fixed effects and replications as random effects. Infection severity values were transformed to arcsine of the square root to stabilize the variances

RESULT AND DISCUSSION

Some agronomical characteristics were observed in field experiment showed that growth duration range from 93-105 days; they belong to early mature group according to Vietnam standards.

The plant height from 167-169 cm. Yield components as ear length from 9.3 to 16.8 cm, diameter from 2.6 to 3.8 cm, number of kernel row/ ear range from 10 to 16 rows and from 8 to 25 kernel /row. Grain yield of inbred lines from 1.13 to 3.72 t/ha, of which there 5 lines with high yield above 3.0 t/ha are D_1, D_5, D_{10}, D_{15}, D_{19}. These are potential lines for develop single cross in maize. Natural tolerance in the field with abiotic and biotic stress as root and stalk lodging, stalk boder **(**Papaipema nebris) , Maydis leaf blight (*Helminthosporium maydis)*, stalk and ear rot (*Fusarium*) showed that scores for natural infection in slight level, the stalk and ear rot infection from level 1 to 3 score and difference among inbred lines (table 2). In terms of artificial *Fusarium* stalk rot infection, significant differences among inbred lines were obtained. All inbred lines showed low level of stalk rot infection regardless of the resistance of lines. Base on the internode is discoloured after inoculated showed that all lines with slight successive level with 4 isolates, it was ranged from 1 to 3 scores correspond to 10 to 75% internode was discoloured.

There are 7 lines were performances resistance to 4 isolates in the greenhouse experiment of artificial Fusarium stalk rot infection are D_1, D_4, D_{10}, D_{14}, D_{15}, D_{16} and D_{20} inbred line, its correspond with 25-50% internode is discoloured, whereas the D_{13}, D_{23} and D_{24} successive to isolate 1 of stalk rot in range 5-6 score correspond with 100.9-156.5% internode and surrounding node are discoloured. The D_{19} line is resistant to isolate 4 at level 1 score, middle resistantto to isolate 3. Although some authors did not find the same symptoms after artificial and natural stalk rot infection (Koehler, 1960), we have found a significant correlation between the results of natural and artificial infection in all inbred lines.The objectives of this study were to identify quantitative trait loci (QTL's) associated with resistance to *Fusarium verticillioides* and evaluate the possibilities of utilizing these QTL's for marker assisted selection (MAS).

A major QTL was identified on chromosome 3, the additive and dominance effects (Jun-Qiang Ding et al, 2008). In this study, we also detected QTL in the 24 inbred lines and showed that the primer *umc 1742* obtained 21 bands and 3 lines without are D13, D16 and D24 (Figure 1). Primer *umc1025* detected on the 24 of maize inbred lines was obtained 19 band have size 150 bp and 5 lines without are D_{13}, D_{18}, D_{22}, D_{23} and D_{24} inbred line. This result suggested that 6/24 lines was not contain QTL control resistance to *Fusarium* stalk rot that are D_{13}, D_{16}, D_{22}, D_{23} and D_{24}. Combined results from evaluation on the field, artificial inoculation and molecular were identified 16 maize inbred lines resistantto to *Fusarium* stalk rot. Resistance genotypes can be utilized in hybrid maize breeding programs for improvement of hybrid maize varieties and development novel germplasm of resistance maize breeding. The correlation between artificial inoculation and field infection were significant level ($r = 0.85$; $P < 0.01$) in spring, respectively This result showed that artificial inovation method can be to sreen and select the maize inbred lines for *Fusarium* stalk rot resistance, Sajjad Hussain Qureshi et al. (2015) also found the same results of this correlation when assessment by two methods on the field and artificial infection. The correlation coefficients of disease scores after natural and artificial infection were in inbreds significant suggest that Fusarium stalk rot resistance should be tested in inbred lines for maize breeding programs.

Table 1. The Origin and pedigree of maize inbred lines

Code No.	Inbred lines	Origin	Pedigree	Code No.	Inbred lines	Origin	Pedigree
1	D_1	USA	S_4	13	D_{13}	Locally developed	S_8
2	D_2	USA	S_5	14	D_{14}	Locally developed	S_7
3	D_3	China	S_5	15	D_{15}	Locally developed	S_8
4	D_4	China	S_5	16	D_{16}	Locally developed	S_8
5	D_5	China	S_5	17	D_{17}	Locally developed	S_7
6	D_6	China	S_5	18	D_{18}	USA	S_5
7	D_7	USA	S_4	19	D_{19}	USA	S_5
8	D_8	China	S_5	20	D_{20}	USA	S_4
9	D_9	China	S_5	21	D_{21}	USA	S_4
10	D_{10}	China	S_5	22	D_{22}	China	S_6
11	D_{11}	Locally developed	S_7	23	D_{23}	China	S_5
12	D_{12}	Locally developed	S_7	24	D_{24}	China	S_5

Table 1: Some agronomical characteristic and yield of 24 maize inbred lines in spring season 2016 at Gialam, Hanoi

Lines	Growth duration (days)	Plant height (cm)	Ear length (cm)	Ear diameter (cm)	No. kernel row/ear	No. kernel/row	Grain yield (t/ha)
D_1	97	147.3	12.4	3.5	13.8	23.1	3.54
D_2	101	157.8	13.8	2.7	14.4	12.5	2.28
D_3	99	144.7	9.9	3.8	13.4	20.9	3.39
D_4	101	159.6	13.5	3.3	13.6	16.1	2.35
D_5	103	160.5	11.5	3.0	13.2	14.8	2.42
D_6	103	167.5	11.5	3.1	13.0	15.2	2.03
D_7	99	166.3	11.0	3.2	13.4	10.8	2.15
D_8	102	156.0	13.7	2.3	14.6	11.2	2.62
D_9	96	147.0	12.1	2.8	13.2	13.3	2.25
D_{10}	100	132.0	12.2	4.2	13.0	24.5	3.57
D_{11}	97	158.0	11.7	3.3	12.8	15.1	2.12
D_{12}	98	166.5	12.5	3.2	12.4	11.5	2.06
D_{13}	103	147.2	9.6	2.8	12.6	7.6	1.13
D_{14}	97	138.0	11.9	3.4	13.4	21.9	2.70
D_{15}	95	168.2	11.3	3.6	11.8	16.9	3.10
D_{16}	96	155.5	12.6	3.5	12.2	15.2	2.64
D_{17}	98	174.2	13.4	3.1	11.8	11.4	18.6
D_{18}	103	147.3	12.6	2.7	12.2	11.7	1.70
D_{19}	96	162.0	16.8	3.7	12.8	22.4	3.73
D_{20}	102	154.6	12.1	3.0	11.0	18.0	2.56
D_{21}	103	168.3	11.7	3.6	11.2	17.8	2.97
D_{22}	101	155.0	10.0	3.2	13.6	9.9	1.72
D_{23}	105	146.5	10.0	2.9	12.8	9.2	1.33
D_{24}	110	158.3	16.6	2.4	12.8	9.6	1.52
cv%	*6.2*	*7.1*	*5.6*	*5.1*	*6.0*	*5.7*	*7.2*
$LSD_{.05}$	*0.9*	*2.3*	*1.0*	*0.5*	*0.3*	*0.9*	*1.0*

Table 2. Lodging tolerance and reaction of maize inbred lines to natural Fusarium stalk rot and leaf blight infection in the field, spring 2016

Lines	Root lodging (%)	Stalk lodging (%)	Stalk border (score)	leaf blight (score)	Stalk rot (score)	Ear rot (score)
D_1	6,2	-	3	-	1	1
D_2	1,7	-	2	1	1	2
D_3	19,5	-	3	1	1	1
D_4	17,6	-	2	-	1	1
D_5	-	-	2	-	1	1
D_6	-	-	3	1	1	2
D_7	12,8	-	3	-	1	1
D_8	3,3	-	2	1	1	1
D_9	-	-	1	-	1	1
D_{10}	1,7	-	2	-	1	2
D_{11}	-	-	2	2	1	1
D_{12}	5,7	-	3	-	1	1
D_{13}	15,5	1,6	4	3	3	2
D_{14}	2,3	-	2	-	1	1
D_{15}	1,5	-	3	1	1	1
D_{16}	7,1	-	3	4	1	2
D_{17}	1,7	6,2	3	4	2	2
D_{18}	3,5	9,2	4	4	3	3
D_{19}	-	-	-	-	1	1
D_{20}	8,8	-	3	2	1	1
D_{21}	9,4	-	4	-	3	1
D_{22}	2,6	-	3	4	2	3
D_{23}	1,3	-	5	2	2	3
D_{24}	-	-	4	1	1	2

Table 3. The inbred lines were performance resistance level to stalk rot isolates by artificial *Fusarium* stalk rot infection in greenhouse artificial inoculation

Line	Isolate 1		Isolate 2		Isolate 3		Isolate 4	
	% discolored	Score	% discolored	Score	% discolored	Score	% discolored	Score
D_1	26.3	2	26.0	2	25.0	2	25.6	2
D_2	55.5	3	28.8	2	30.4	2	23.3	2
D_3	51.7	3	23.6	2	32.0	3	23.5	2
D_4	27.5	2	26.3	2	25.8	2	28.1	2
D_5	25.9	2	30.5	2	52.3	3	30.5	2
D_6	52.3	3	59.2	3	55.6	3	25.4	2
D_7	56.7	3	52.5	3	57.2	3	65.8	3
D_8	29.3	2	54.0	3	29.5	2	29.8	2
D_9	28.6	2	30.0	2	56.4	3	29.6	2
D_{10}	25.2	2	27.1	2	27.2	2	28.0	2
D_{11}	51.1	3	58.3	3	51.3	3	29.9	2
D_{12}	26.3	2	25.0	2	27.9	2	29.1	2
D_{13}	156.5	6	40.1	3	30.3	2	30.7	2
D_{14}	25.5	2	26.2	2	26.3	2	27.8	2
D_{15}	29.3	2	23.0	2	27.5	2	26.9	2
D_{16}	27.5	2	25.9	2	27.7	2	25.7	2
D_{17}	33.5	2	55.1	3	35.9	3	47.9	2
D_{18}	75.0	3	44.9	3	65.5	3	56.7	3
D_{19}	10.2	2	25.0	2	23.4	2	4.5	1
D_{20}	27.8	2	25.3	2	34.7	2	29.2	2
D_{21}	54.2	3	29.7	2	31.6	3	52.5	3
D_{22}	38.5	2	74.2	3	65.8	3	67.3	3
D_{23}	101.3	5	30.0	2	70.9	3	63.5	3
D_{24}	100.9	5	30.2	2	65.5	3	69.7	3

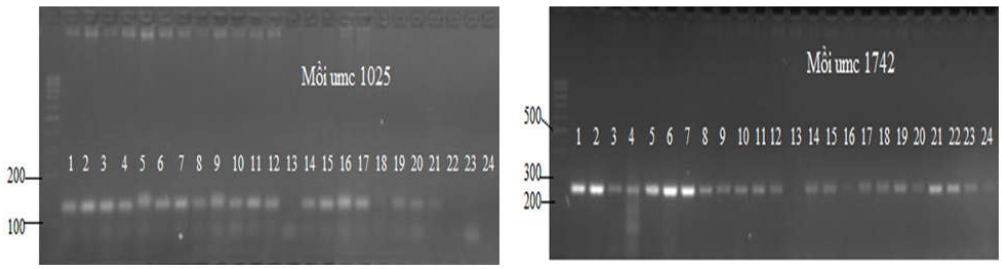

Figure 1. PCR-SSR products with primer *umc 1742* and *umc 1025* detected in 24 maize inbred lines

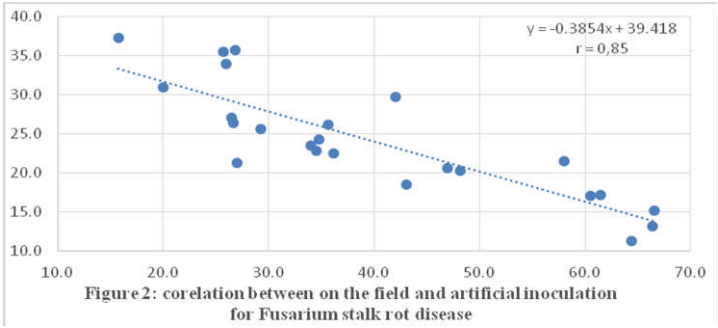

Figure 2: corelation between on the field and artificial inoculation for Fusarium stalk rot disease

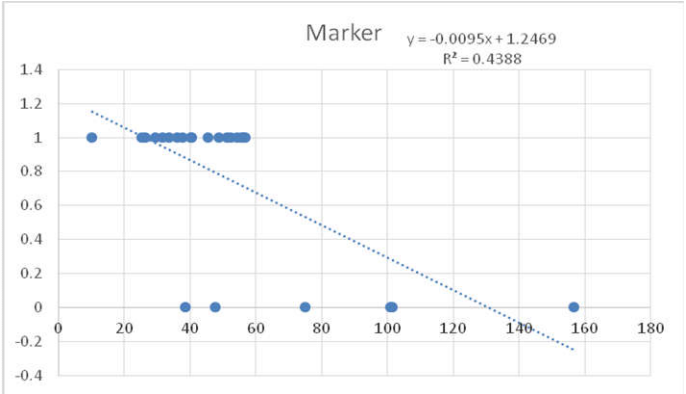

Figure 3: Correlation between detected the resistance QTL by SSR marker and evaluated by artificial infection to *Fusarium* stalk rot

Corelation between molecular marker analysis and artificial infection evaluation also at significant level ($r = 0.438$; $P < 0.01$) as figure 3. This result was recommended can use molecular marker to screen inbred lines for maize breeding resistance to *Fusarium* stalk rot.

Conclusion

Our results suggested that testing for *Fusarium* stalk rot resistance needs to be conducted in inbred lines. However, the use of resistant parental lines led generally to resistant hybrids (Sajjad Hussain Qureshi *et al.,* 2015). Artificial stalk rot infection followed by a natural infection test can be recommended in maize resistance breeding. Twenty-four maize inbred lines showed the appropriated agronomical characteristics and grainyield of inbred lines from 1.13 to 3.72 t/ha, of which there 5 lines with high yield above 30 t/ha are D_1, D_5, D_{10}, D_{15}, D_{19}. These are potential lines for developing single cross in maize. Natural infection, artificial inoculation combined molecular marker identified 18 inbred lines resistantto to *Fusarium* stalk rot are D_1, D_2, D_3, D_4, D_5, D_6, D_7, D_8, D_9, D_{10}, D_{11}, D_{12}, D_{14}, D_{15}, D_{17},D_{19}, D_{20} và D_{21}, these lines can be utilized to develop the maize hybrids resistance to *Fusarium* stalk rot in Vietnam

REFERENCES

Afolabi CG, Ojiambo PS, Ekpo EJA, Menkir A, Bandyopadhyay, R 2008. Novel sources of resistance to *Fusarium stalk* rot of maize in tropical Africa. Pl Dis 92: 772–780.

De Leon C., S. Pandey, 1989. Improvement of resistance to ear and stalk rots and agronomic traits in tropical maize gene pool. Crop Sci. 29: 12-17.

FAOSTAT, 2014 (http://faostat3.fao.org)

Hoisington D, Khairallah M, Gonzalez-de-Leon D. 1994. Laboratory protocols: CIMMYT applied molecular genetics laboratory, 2nd edn. CIMMYT, Mexico, DF

Jun-Qiang Ding, Xiao-Ming Wang, Subhash Chander, Jian-Bing Yan,Jian-Sheng Li, 2008.QTL mapping of resistance to Fusarium ear rot using a RIL population in maize, Mol Breeding, DOI 10.1007/s11032-008-9184-4

Meena Shekhar & Sangit Kumar, 2012, Inoculation Methods And Disease Rating Scales For Maize Diseases, Indian Council of Agricultural Research, Pusa Campus, New Delhi – 110 012 (India), Second Edition, 2012 (Revised)

Missmer, S.A., L. Suarez, M. Felkner, E.Wang, A.H. Jr. Merrill, K.J.Rothman and K.A.Hendricks, 2006. Exposure to fumonisins and the occurrence of neural tube defects alog the Texas- Mexico border. Environ. Health Perspec., 114: 237-241

Morgavi, D.P. and R.T. Riley, 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. Anim. Feed Sci. Technol., 137: 201 – 212

Prelusky, D., B. Rotter and R. Rotter, 1994. Toxicology of Mycotoxins. In: Mycotoxins in Grain: compounds other than Aflatoxin, Miller, J. and H. Trenholm (Eds.). Eagan Press, St. Paul, pp: 359-403

Sajjad Hussain Qureshi, abdul qayyum, Will FIERS, 2015. Sources of genetic resistance in maize to Fusarium stalk rot and their variations at molecular level, Turk J Agric For (2015) 39: 503-513

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