DEVELOPMENT OF RESISTANT MAIZE GERMPLASM

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ABSTRACT:- During spring 1999, on the basis of survived plants, a total of 250 accessions including 150 exotic out of 350 and 100 indigenous out of 900 were selected. During autumn 1999, out of 250 accessions (progenies) 35 exotic and 10 indigenous could survive under artificial infestation. Under natural infestation, 100 progenies performed little better. The best plants among these progenies were advanced by self pollination. A total of 400 self pollinated cobs (as progenies) were screened during spring 2000 under natural infestation. The borer attack started at a very initial stage of the crop and swept down many of the progenies. Only 88 exotic and 7 indigenous progenies showed some resistance. The survived plants, among these progenies, were advanced by self pollination. During autumn 2000, only 9 progenies out of 95 showed some resistance with 35-66% plants survived under artificial infestation. Under natural infestation, 10 other progenies had more than 50% survived plants. The best survived plants among these 19 progenies were advanced by selfing. During spring 2001, 8 of the comparatively resistant germplasm showed no leaf damage while 9 others had 7.10 - 44.7% infestation. In checks, the infestation was from 27.9 to 100.0%. The selected materials were again maintained by self pollination to make pure lines. During autumn 2001, selected material was screened under double artificial infestation, first at 3-4 leaf stage of the crop and the second 10 days after first infestation with 15 newly hatched larvae per plant every time. Despite of double artificial infestation, 40.0-66.7% plants survived in the resistant germplasm but none survived in susceptible genotypes. Under natural infestation, 5 progenies had no damage while other showed 10.0-22.9% damage with intensity of 2-3 in resistant germplasm. The susceptible germplasm showed 30.0-66.6% infestation with intensity of 5-8. The dead hearts in resistant and susceptible germplasm were 0.0-7.8% and 19.6-25.4%, respectively. The performance of the resistant germplasm was far better than susceptible ones. The best plants in resistant germplasm were maintained by selfing to make them more uniform for resistance. During pollination, three resistant composite genotypes (BR-1, BR-2 and BR-3) were constituted by bulk pollination.

Key Words: Zea mays; Stem Borer; Chilo partellus; Damage; Screening; Resistant Germplasm; Pakistan.

INTRODUCTION

Maize *(Zea mays L.)* being the highest yielding cereal crop in the world, is of significant importance for countries like Pakistan, where rapidly increasing population and poultry industry have already out stripped the available food and grain supplies. Maize crop possesses great

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genetic diversity and can be grown across varied agro-ecological zones (Ferdu et al., 2002). In Pakistan, maize is being grown on 0.935 mha with annual grain production of 3.262 mt and average grain yield of 3488 $kgha^{-1}$ (FAO, 2011). It is the third most important cereal after wheat and rice. Among several factors responsible for low yields, significant contribution has been made by insect pests. Maize stem borer *(Chilo partellus)* is the most notorious pest that causes heavy damage to maize crop resulting from 10 to 100% yield losses in Asia and Africa (Bergvinsion et al., 2004; Songa et al., 2002). According to Zhou-GuoFa (2001), C. partellus was found at all locations of Kenya with elevations up to 2300 m with its highest density in the semi-arid ecological zone.

Mainly, four major components of integrated pest management viz., chemical, biological, host plant resistance, and cultural methods are being used for the control of insects in the world. In Pakistan, most of the farmers use pesticides for insect control while very few farmers use biological control method. It is very difficult to establish biological control mean every where. Use of insecticides is the most effective and quick method of insect control but have so many adverse effects like mortality of biological control agents, environmental and water pollution, and biohazard to human beings and animals. He et al. (2004) and Abel et al. (2000) mentioned that host plant resistance is an important com-ponent of integrated manage-ment of borers and several other insect pests and can effectively maintain pest populations below economic threshold levels, especially in low-input subsistence farming system. The technology regarding maize germplasm resistant to insect pests can be the sustainable, low cost, economical, more productive and free from any environmental pollution and other biohazards. The phenomenon of plant resistance to insects is a quality that enables a plant genotype to avoid, tolerate, or recover from the effects of oviposition or feeding that would cause greater damage to other genotypes of the same species under similar environmental conditions. According to Lynch et al. (2003), host-plant resistance is an efficient, economical and environmentally compassionate approach used to manage many pests and diseases of agricultural crops. In Pakistan, *C. partellus* attacks the crops at very early stage (about 4-5 leaf stage of plants) and turns the plants to dead hearts. Therefore, it is necessary to develop and test the resistance in maize at an early stage. There may be yield penalty resulting from the diversion of the plant resources to resistance mechanisms.

Keeping in view the risks and difficulties involved in chemical and biological control of insects, resistant maize germplasm becomes essential in a country like Pakistan. Present studies were, therefore, designed to develop resistant maize germplasm against *C. partellus*.

MATERIALS AND METHOD

For maintenance of culture of maize stem borer (*C. partellus*), the larvae were reared on media described by Siddiqui and Chatterji (1972) and Ochieng et al.

(1985). Media, described by them was slightly modified by adding maize tassel powder (Table 1). For development of resistant source, about 350 accessions of maize germplasm having some resistance against *Diatraea grandiosella* were collected from exotic sources through International Maize and Wheat Improvement Centre (CIMMYT) Mexico and about 900 germplasm from indigenous sources. At CIMMYT Mexico, USA and in many other countries, the borer attacks maize at knee height of the crop and selection for resistance against first brood has been done at that stage. In Pakistan, the first brood attacks the maize crop at early stage (3-4 leaf stage) of plant growth.

Table 1. Ingredients of medium used for rearing *Chilo partellus*

Factor A	
Agar Agar	10.2 g
Water	400.0 ml
Factor B	
Evion	1.0 g
Sorbic acid	1.0 g
Ascorbic acid	2.5 g
Methyl parabin	1.6 g
Yeast	8.0 g
Kidney Bean powder	110.0 g
Sugarcane leaf powder	30.0 g
Maize tassel powder	10.0 g
Water	450.0 ml
Formaldehyde	2.0 ml

All the germplasm were screened and advanced for resistance under artificial and natural infestations at the early stage of the crop at National Agricultural Research Centre, (NARC) Islamabad, Pakistan. The screening and advancement was done for six seasons from spring 1999 to autumn 2001. Each year during spring season, screening was done under naturally occurring populations of *C. partellus*, and during autumn under artificial infestations. A set of available improved varieties were also planted as negative checks. For this purpose, four 5m long rows, with 75 cm row to row and 25 cm plant to plant distance were used. Thus 20 plants in one row were grown. During autumn, 15 newly hatched larvae were released by a fine camel's hair brush in plant whorl of each of the 10 plants of each progeny at 3-4 leaf stage. Among the remaining 10 plants, 6 were kept under natural infestation and 4 were protected by using insecticide. Protected plants were healthy and showed complete vigour of the germplasm for comparison as positive check. For improvement and advancement of resistance in selected germplasm, self pollination method of breeding was used. Initially during spring 1999, selection was made on the basis of plant survival. The remanent seeds of the selected progenies were further screened for resistance under natural and artificial infestation during autumn 1999. The survived plants were advanced by self pollination. The seeds from the advanced ears of selected progenies were planted ear-to-row during spring 2000 for testing and improvement in comparison with 10 existing susceptible genotypes under natural infestation of *C. partellus*. The healthy plants were advanced further through self pollination. The seed of the selfed ears of respective progenies were mixed to maintain the genetic vigour. The selected progenies were planted and screened under artificial infestation during autumn 2000 and advanced the selected plants through self pollination. Again, the selected ears of the selected progenies were advanced during spring 2001 under natural infestation.

Almost the same germplasms

were tested and screened under double artificial infestation of *C. partellus* during autumn 2001. First infestation was done at 3-4 leaf stage of the crop and the second infestation was done 10 days after first infestation with 15 newly hatched larvae per plant every time. The data regarding survival of plants and extent of damage in these plants were recorded. Intensity was observed on scale described by Ampofo and Sexena (1989). Dead hearts formed were also counted as it is a good indicator for resistance at early stage of the crop as described by Mihm (1989). The best plants were advanced through self pollination. Three composite genotypes (BR-1, BR-2 and BR-3) were formed through bulk pollination.

RESULTS AND DISCUSSION

During spring 1999, on the basis of survived plants, a total of 250 accessions including 150 exotic out of 350 and 100 indigenous out of 900 were selected. During autumn 1999, the selected 250 accessions (progenies) were screened under natural occurring and artificial infestation. Majority of the plants could not tolerate this artificial stress and died. A few plants including 35 exotic and 10 indigenous progenies survived. Though exotic germplasm had some resistance, even then majority of them could not show resistance at 3-4 leaf stage. Under natural infestation, 100 progenies performed little better. The best plants among these progenies were advanced by self pollination. A total of 400 self pollinated cobs (as progenies) were screened during spring 2000 under natural infestation. The borer attack started at very initial stage of the crop and swept down many of the progenies. Only 88 exotic and 7 indigenous progenies showed some resistance. The survived plants, among these progenies, were advanced by self pollination. The seed of the selfed ears of respective progenies were mixed to maintain the genetic vigour. During autumn 2000, the 95 selected progenies were screened for further improvement and tested in comparison of 5 improved cultivars. Only 9 progenies showed some resistance (Table 2) and 35-66% plants survived under artificial infestation. Under naturally prevailing population of stem borer, 19 progenies had more than 50% survived plants. The best survived plants among these 19 progenies were advanced by selfing. The best ear from each selected progeny was planted for testing in comparison with 10 improved cultivars and for advancement during spring 2001.

The data regarding leaf, tassel and stem damages under natural infestation of *C. partellus* during spring 2001 (Table 3) showed the performance of progenies.

Initially (20 days after germination), 11 resistant progenies showed no damage while 5 had 1.55 - -5.55% infested plants. In improved varieties (the susceptible checks), 18.1-48.1% plants got infested. Forty days after germination, infestation came from second brood of *C. partellus*. Even then, 8 of the comparatively resistant germplasms showed no leaf damage while 9 others had 7.10 - 44.7% infestation. In checks, the infestation increased significantly from 27.9 to 100.0%.

Table 2. Screening of maize germplasm for resistance against *Chilo partellus*, under natural and artificial conditions at NARC, Islamabad during autumn 2000.

E/No.	Germplasm	Survival (%)		E/No.	Germplasm	Survival (%)	
		Under artificial infestation	Under natural infestation			Under artificial infestation	Under natural infestation
NARC-25							
1	NARC-25-1-1*	40	60*	5	NARC-25*	33.33	50*
2	NARC-25-6-3-3	0	30	6	NARC-25 F1*	0	66.67*
3	NARC-25-6-3-4	0	0	7	NARC-25 F1 R*	33.33	50*
4	NARC-25-12-1*	20	50*	8	NARC-25 F1 Ch	0	10
TL 97A POP-590 C5 AM S3 1751 A							
9	1-2	0	0	27	50*	0	40
10	18	0	0	28	54-2*	0	80
11	21-1	0	20	29	67-1*	0	35
12	24-1	0	28.57	30	70-2	0	20
13	24-3	0	0	31	79-1	0	25
14	25-1	0	0	32	79-2*	0	33.33
15	35-3	0	0	33	82	0	25
16	89	0	0	34	149	0	14.28
17	98-1	0	0	35	155	0	16.67
18	106-1	0	0	36	157-1	0	0
19	117	0	0	37	181-3	0	0
20	118-2	0	0	38	182	0	0
21	119-2	0	0	39	192-1	0	0
22	119-3	0	0	40	202-1	0	0
23	120-1	0	16.67	41	206-1	0	0
24	131-1	0	0	42	210	0	0
25	137-2*	0	33.33	43	222	0	0
26	147-3*	0	75*		223*	0	33.33
TL 97A POP-590 MBR C5 AM 1752 A							
44	10-1	0	0	53	43-3*	0	85*
45	14-2*	20	80*	54	44-2*	0	65.56*
46	16-1*	0	50	55	56-2*	0	33.33
47	16-2*	66.67	80*	56	57-1	0	20
48	17-3*	50	60*	57	57-3	0	28.57
49	20-2*	0	33.33	58	62-4	0	28.57
50	23-2*	0	80*	59	71-1	0	28.57
51	31-2	0	0	60	71-4*	0	50
52	43-2*	0	87.5				
TL 97B POP-590 MBR/ETLIN. ELIT C1 AM 6765 A							
61	6*	0	26.89*	74	37	0	0
62	9*	20	88.89*	75	40*	10	60.67*
63	11*	10	60*	76	41*	0	50*
64	12*	0	35.5	77	51	0	20
65	13*	0	37.14	78	54*	5	62.86*
66	14*	0	33.33	79	55*	0	50
67	15*	0	56.67	80	56*	0	50
68	18*	0	55.6	81	57*	0	46
69	22	0	0	82	59	0	20
70	23*	0	33.33*	83	61	0	25
71	24*	0	50*	84	70	0	25
72	28*	0	38*	85	72	0	0
73	29*	0	63.33*	86	73*	0	31.43
CHECK VARIETIES							
87	Margala	0	0	90	EV-2097	0	0
88	Gauher	0	0	91	POP-9746	0	0
89	EV-1085	0	0				

* *Selected germplasm*

Due to heavy lodging, stem damage could not be recorded. At tasselling stage, adults of *C. partellus* laid eggs on flag leaves. The larvae, on emergence, feed on tassels. In resistant germplasm, 3.33 to 25.0% and in checks 10.0 - 41.67% of the plants had infested tassels. The materials with high infestation were rejected. The selected materials were again maintained by self pollination to make pure lines.

During autumn 2001, under double artificial infestation, 40.0 - 66.7% of the plants survived in the resistant germplasm but none survived in susceptible genotypes (Table 4).The intensity of damage in resistant germplasm was 3-4 on standard 0-9 scale. In the susceptible germplasm, the intensity was very high (8-9 scale). Under natural conditions of infestation, 5 progenies had no damage while other showed 10.0-22.9% damage with intensity of 2-3 in resistant germplasm. The susceptible germplasm showed 30.0-66.6% infestation with intensity of 5-8. The dead hearts in resistant and susceptible germplasms were 0.0-7.8 and 19.6-25.4%, respectively. During early attack of stem borer, dead hearts formation is an important criterion to observe. The performance of the resistant germplasm was far better than susceptible ones. The best plants in resistant germplasm were maintained by selfing to make them more uniform for resistance. During pollination, three resistant composite genotypes were constituted by bulk pollination. BR-1 was constituted among the best plants of 25 F, 25-1-1 and 25-12-2, BR-2 among the best plants of 1751-54-2, 1752-44-2, 1752-43-3, 1752-

Table 3. Screening of maize germplasm for resistance against *C. partellus* under natural conditions, at NARC, Islamabad during Autumn 2001

E/No.	Entries	Leaf damage (20 DAG) (%)	Leaf damage (40 DAG) (%)	Tassel damage (%)	E/No.	Entries	Leaf damage (20 DAG) (%)	Leaf damage (40 DAG) (%)	Tassel damage (%)
1	25 1-1	5.55	21.65	7.94	16	C 6765-41	0.00	0.00	14.29
2	25 F1	3.30	7.10	3.33	17	C 6765-54	0.00	0.00	7.14
3	C 6765-6	0.00	0.00	4.35	18	C 1752-14-2	0.00	0.00	14.55
4	C 6765-9	0.00	25.00	15.79	19	C 1752-16-2	0.00	0.00	8.33
5	C 6765-11	0.00	14.30	13.33	20	C 1752-17-3	0.00	0.00	6.67
6	C 6765-23	5.60	10.00	5.56	21	C 1752-23-2	6.50	15.00	6.45
7	C 6765-24	1.55	10.00	9.90	22	C 1752-43-3	2.15	16.65	11.70
8	C 6765-28	0.00	33.30	40.00	23	C 1752-44-2	13.05	44.70	23.34
9	C 6765-29	0.00	11.10	11.11	24	C 1751-147-3	8.60	25.00	8.57
10	C 6765-40	0.00	0.00	25.00	25	EV-2097	16.15	38.15	15.84
11	Kissan	29.20	100.00	41.67	26	EV-1097	22.65	27.90	14.04
12	SN-2000	22.50	38.90	10.00	27	Sarhad Y	29.55	47.05	23.87
13	Margala	18.10	53.80	18.75	28	Agaiti-85	48.10	35.70	14.81
14	Magic	43.30	75.00	26.67	29	Ghouri	45.50	57.10	27.27
15	Gauher	22.30	55.00	24.29					

DAG = Days after germination

Table 4. Screening of maize germplasm for resistance against *C. Partellus*, Under natural and artificial conditions at NARC, Islamabad during Autumn 2001

Germplasm	Artificial infestation			Natural infestation	
	Survival (%)	Intensity of damage (0-9)	Damage (%)	Intensity of damage (0-9)	Dead hearts (%)
NARC -25-1-1	61.1	3	0.0	0	0.0
NARC-25 F	70.0	2	0.0	0	0.0
C-6765-40	67.5	3	3.6	2	2.6
C 6765-9	63.3	3	0.0	0	0.0
C 6765-11	50.0	4	7.55	3	3.0
C 6765-28	40.0	4	10.0	2	7.10
C 1751-54-2	51.1	4	0.0	0	0.0
C 1752-14-2	66.7	3	12.0	3	3.50
C 1752-16-2	60.1	4	22.9	3	4.20
C 1752-17-3	64.3	3	0.0	0	0.0
C 1752-43-3	51.1	4	15.0	3	7.80
C 1752-44-2	55.1	3	13.0	3	0.0
C 1751-147-3	43.3	4	0.0	0	5.30
Sarhad yellow	0	9	30.0	5	19.60
Kissan	0	9	66.6	7	26.67
Margala	0	9	46.7	5	22.00
Gauher	0	9	50.0	8	25.00
EV-2097	0	9	60.0	7	22.20
EV-1097	0	9	40.0	7	25.40
Agaiti-85	0	9	50.0	7	24.7

14-2 and 1752-16-2 and BR-3 among the best plants of 6765-9, 6765-11 and 6765-40.

Artificial infestation for screening the germplasm was done because it gives fast and accurate results while natural infestation alone was not effective, very slow and the results could be misleading. The infestation with neonate larvae resulted in higher foliage damage and greater dead hearts (Jindal and Hari, 2010). Most of the entomologists are convinced that artificial infestation is superior and more efficient than other techniques for identifying resistant genotypes. Conventional breeding techniques have facilitated significant improvement in resistance to corn borers. Siddiqui et al. (1996) evaluated the varieties for resistance on the basis of only leaf injury. Kumar et al. (1993) evaluated the varieties for resistance on the basis of leaf and stem feeding and dead hearts. Sharma and Sharma (1992) identified the resistance in plants on the basis of lowest leaf damage. Khaliq and Mahmood (1991) selected the resistant variety with 17.7 % infestation. Mihm (1989a) also suggested that resistance should be measured by the absence or low percentage of dead hearts when borers attack the plants at seedling stage. In the present studies, comprehensive criteria of resistance including leaf, stem, tassel and ear damages, dead hearts formation and survival of plants were followed.

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