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Effect of Cultivar Mixture on Epidemic Development of Stripe Rust (*Puccinia striiformis F.sp. Tritici*) and Yield of Bread Wheat in Southeastern Ethiopia

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Abstract: Stripe rust is a major treat to wheat production in Southeastern highlands of Ethiopia resulting in high yield and quality losses. Field experiments were conducted to study the effect of seven cultivars and their mixtures in various proportions (1:1, 1:3 and 3:1) on the epidemics of stripe rust and yield of bread wheat under natural epiphytotics. The experiments were conducted in the main seasons (August-December) of 2004 and 2005 at three stripe rust prone areas of southeastern Ethiopia (Sinana, Hisu and Selqa). Stripe rust severity levels and AUDPC values in most of the mixture combinations were significantly reduced as compared to the pure stands of the susceptible components, invariably with test locations and years of experimentation. Depending on the test locations and years, terminal stripe rust severity reached 40-85, 7-34, 32-79, 10-55.8, 4-17.5, 20-50 and 18-79.3% in pure stands of the varieties Wabe, Dure, Kubsa, Sofumer, Mada walabu, K6295-4A and Mitikie. Mixtures provided average mixture efficacy and reduction in AUDPC by 26.2 and 23.0%, respectively. In thirteen of the twenty mixtures in 1:1 proportions, the yield obtained was greater than the expected mixture yield by an average value of about 8.6%. Yield exceeding that of the best yielding component was recorded only for mixtures between Sofumer-Kubsa and Mitikie-K6295-4A in equal proportions, with average percent yield advantage of 4.7 and 5.3 than the pure stands of Sofumer and K6295-4A, respectively. In all the cases of 3:1 proportions of resistant: susceptible cultivar mixtures, actual yield was greater than the expected mixture yield of the respective components. Mixture provided yield lower than the low yielding component only in one case, *i.e.* in the mixture between Mada Walabu-K6295-4A at Hisu in 1:1 proportions.

Key words: Stripe rust % Epidemics % Cultivar mixture % AUDPC and yield

INTRODUCTION

Bale and Arsi highlands in the southeastern Ethiopia are the major bread wheat producing regions and are deemed the wheat belt of east Africa. Stripe rust caused by *Puccinia striiformis f.sp. tritici* is a major treat to wheat production in the region resulting in high yield and quality losses [1,2]. Grain yield losses in the range of 30 to 96% have been recorded on susceptible bread wheat cultivar [3-5]. In southeastern Ethiopia wheat production is characterized by continuous and extensive monoculture of varieties with narrow genetic bases. Wheat is also produced twice a year, during main season (August-December) and the short season (March-July) [1]. This situation provides the pathogen not only with a substrate both in area and time, but also results in successive evolution of pathotype lineages and/or new races of the pathogen [6, 7].

The use of resistant cultivars has been the major strategy to control stripe rust of wheat in Ethiopia. Many high yielding and rust resistant bread wheat varieties have been released at national and regional levels in the country. The resistance in many of such cultivars has generally been short lived and easily overcome by mutation and selection of virulent pathotypes resulting in the historical boom and bust cycle of rust epidemics and losses in wheat yield. The cost of fungicides on the other hand may not be affordable to subsistent farmers. As a

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result, alternative strategy has been investigated through the use of genetically varied host cultivar mixture which provides a significant level of disease restriction [8, 9]. The use of cultivar mixtures has been demonstrated to reduce disease severity, rate of pathogen evolution and improve yield [7, 10, 11]. Cultivar mixture could be more important in eastern Africa where frequent evolution of highly specialized pathogens, the wheat rusts, is a serious challenge to the global wheat production. In spite of enormous advantages of cultivar mixture however, little or no work has been done to evaluate the effect of cultivar mixture on the epidemics of stripe rust of wheat and the associated yield changes in Ethiopia. Thus, the purpose of this study was to investigate the efficacy of different proportions of bread wheat cultivar mixture on the epidemics of wheat stripe rust and yield and yield components of bread wheat in the highlands of Bale.

MATERIALS AND METHODS

Experimental Site: The experiments were conducted at three rust prone locations of Bale highlands: Sinana, Hisu and Salqa during the main seasons (August - December) of 2004 and 2005. Those locations represent the major wheat growing areas of the country and are characterized by high rainfall with regular stripe rust epidemics. They are located at an altitude of 2320-2450 meters above sea level (masl) in the southeastern part of Ethiopia. The soils type is predominantly pellic vertisol, which is slightly acidic. There are two rainy seasons per annum, main season (August-December) and the second season (March-July), during which wheat is extensively produced at almost equal proportion. The locations receive an average annual rainfall of about 450 mm during the main with average minimum and maximum temperature is 9.4 and 21.2°C, respectively.

Treatments, Experimental Design and Field Plot: Seven bread wheat cultivars were used to study the efficacy of variety mixture on the epidemic development of stripe rust and yield of wheat under natural epyphtotics. In 2004, five one-way cultivar mixtures (in 1:1 proportion) were used in addition to the pure stands of the cultivars. This constituted a total of twelve treatments. In 2005, ten two-way cultivar mixtures (1:3 and 3:1) were used in addition to the 1:1 mixture proportion and pure stand the cultivars which constitute a total 22 treatments. The locations were Sinana and Hisu in 2004; and Sinana and Selqa in 2005. The component cultivars were selected on the basis of their differential reaction to stripe rust and similarity in agronomic traits; days to maturity, plant height, seed color and other agronomic performances. The cultivars used were Kubsa, Wabe, Dure, Sofumer, Mada Walabu, K6295-4A and Mitikie. Kubsa and Wabe were highly susceptible to stripe rust while Mitikie, Sofumer and K6295-4A are moderately susceptible. Dure and Mada Walabu are moderately resistant and highly resistant, respectively. The mixture compositions were formulated between Kubsa-Dure, Wabe-Dure, Kubsa-Sofumer, Mada Walabu-K6295-4A and Mitikie-K6295-4A. The experiments were laid out in randomized complete block design (RCBD) in factorial arrangement with three replications. The varieties were planted to six rowed plots of 6 m length with a distance of 20 cm between rows. The gaps between plots and replication were 1.5 m and 2 m wide, respectively. Seed rate of 150 kg, which is recommended for the area, was used. Fertilized at a rate of 41 kg/ha N and 46 kg/ha, P_2O_5 were applied during planting. Weeds were controlled by two to three times hand weeding.

Disease Assessment: Stripe rust severity was recorded three times on whole leaves at four randomly selected spots in 1-m long row plot using the modified Cobb's scale [12]. The average stripe rust severity from the four spots of each plot was used for analysis and inputs for area under the disease progress curve (AUDPC) determination and disease progresses analysis. AUDPC values were calculated using the following formula [13]:

$$AUDPC = \sum_{i=1}^{n-1} 0.5(x_{i+1} + x_i)(t_{i+1} - t_i)$$

Where, x_i is the cumulative disease severity expressed as a proportion at the i^{th} observation, t_i is the time (days after planting) at the i^{th} observation and n is total number of observations. Since the duration of assessment was not same for each epidemic, AUDPC values were standardized by dividing the values by the total duration (t_n-t_1) of the epidemic.

Grain Yield: Grains were sun dried and adjusted to about 12.5% moisture content level. Grain yield (gram per plot) that harvested from four internal rows of the plots, excluding 0.5 m from both sides along the length of the plot was converted to gain yield kg/ha and used in analysis.

Statistical Analysis: Data of terminal disease severity and AUDPC values and grain yield were subjected to analysis of variance using Minitab for windows

(Release 12.21). Comparison of treatment means was made using least significant difference test (LSD). Mixture efficacy (ME) was calculated as percentage of reduction of stripe rust severity relative to the pure stands of the susceptible components using the formula:

$$ME(\%) = [(Y_{ps}-Y_m)/Y_{ps}]x100$$

where ME is mixture efficacy, Y_{ps} is stripe rust severity in pure stands of the relatively susceptible component and Y_m is stripe rust severity in the mixture.

RESULTS AND DISCUSSION

Development of Stripe Rust Epidemics: Severe epidemics of stripe rust were developed during both seasons most notably at Sinana. Appreciable levels of stripe rust severity were also recorded at Hisu and Selqa. Depending on test locations and years of experimentation, terminal stripe rust severity was reached 40-85, 7-34, 32-79, 10-55.8, 4-17.5, 20-55 and 18-79.3 % in the pure stand varieties Wabe, Dure, Kubsa, Sofumer, Mada Walabu, K6295-4A and Mitikie, respectively (Tables 1 and 2). Stripe rust severity levels in most of the mixture combinations were significantly (P # 0.05) reduced as compared to the pure stands of the susceptible components, invariably with test locations and years of experimentation. Mixture provided average mixture efficacy of 26% over location and seasons. Mixture efficacy increased consistently with increasing the proportion of resistant cultivar in the mixture formulations.

Mixture between Wabe and Dure in 1:1 proportions provided mixture efficacy in the range of 20-53% average 34% over locations and years, while the mixture treatment in 1:3 and 3:1 proportions resulted in average mixture efficacy of 44 and 16% respectively (Table 1 and 2). In Kubsa- Dure mixture in 1:1, 1:3 and 3:1 proportions, stripe rust severity was contained on average by 38, 50 and 16%. K6295-4A-Madawalabu mixture treatments in 1:1 proportions on the other hand provided average stripe rust reduction of 27% while the 1:3 and 3:1 proportion of this mixture gave mixture efficacy of 56 and 12%, respectively. Stripe rust severity in mixtures between Mitikie and K6295-4A mixture was not significantly reduced in all proportions except at Sinana in 2005 when the 1:3 mixture proportion gave mixture efficacy of about 25% (Table 1 and 2). Levels of stripe rust severity were not significantly affected by the different proportions of the mixture between Kubsa and Sofumer as compared with the pure stands of the susceptible component except at Sinana in 2004 where mixture treatment in 1:3 proportion significantly reduced stripe rust severity by up to 35% (Table 1 and 2).

The results of the present study demonstrated that host mixtures can provide considerable restriction in disease levels compared to the mean of their components. Many epidemiological studies with cultivar mixtures also presented similar findings. Working with rusts and powdery mildew of small grains pathosytems, Mundt and Browning [14] and Wolfe [15] reported that disease severity will decrease with decreasing frequency of a host genotype in the mixture. In appropriate mixtures of

Table 1: Levels of terminal stripe rust severity, mixture efficacy and AUDPC values in pure stands of bread wheat varieties and their mixtures in equal proportions at Sinana and Hisu during the main season of 2004

	Terminal stripe rust severity (%)		Mixture efficacy (%)		Standardized AUDPC (%)	
Treatments	Sinana	Hisu	Sinana	Hisu	Sinana	Hisu
Wabe pure stand	85.0	40.0	-	-	55.9	24.3
Dure+Wabe	40.0	25.0	52.9	37.5	23.3	16.6
Dure pure stand	27.0	7.0	-	-	11.8	4.8
Dure+Kubsa	38.0	24.0	51.8	25	24.5	19.7
Kubsa pure stand	79.0	32.0	-	-	54.9	27.8
Sofumer+Kubsa	51.0	27.0	35.4	16.5	34.2	20.4
Sofumer	21.0	10.0	-	-	11.6	5.8
Madda Walabu	4.0	9.0	-	-	2.3	5.8
Madda Walabu + K6295-4A	32.0	15.0	25.6	25	14.7	9.5
K6295-4A pure stand	43.0	20.0	-		20.5	10.2
Mitike+K6295-4A	39.0	17.0	9.3	15	21.0	8.4
Mitike pure stand	37.0	18.0	-	-	21.2	12.8
SE	3.6	3.0	-	-	2.7	1.4
5% LSD	10.6	8.7	-	-	7.0	6.0

SE=Standard error, LSD = Least Significant Difference

Table 2: Levels of terminal stripe rust severity, mixture efficacy and AUDPC values in pure stands of bread wheat varieties and their mixtures in varies proportions at Sinana and Selqa during the main season of 2005

	Terminal stripe rust severity (%)		Mixture efficacy (%)		Standardized AUDPC (%)	
Treatments	Sinana	Selqa	Sinana	Selqa	Sinana	Selqa
Wabe pure stand	77.5	50.5	-	-	66.8	34.4
25% Wabe+ 75% Dure	51.7	23.5	33.2	53.5	37.8	11.5
50% Wabe + 50% Dure	61.7	37.9	20.4	25.0	44.5	18.7
75% Wabe + 25% Dure	65.0	42.5	16.1	15.8	47.0	26.1
DR pure stand	34.0	17.4	-	-	28.4	15.6
25% Kubsa + 75% Dure	45.7	18.3	34.7	64.0	30.4	14.5
50% Kubsa + 50% Dure	50.0	26.3	28.6	48.2	43.6	20.7
75% Kubsa + 25% Dure	60.8	40.8	13.1	19.7	53.5	32.4
Kubsa pure stand	70.0	50.8	-	-	63.3	40.8
25% Kubsa + 75% Sofumer	57.5	43.7	17.9	14.0	55.3	36.7
50% Kubsa + 50% Sofumer	61.7	45.8	11.8	9.8	63.9	36.5
75% Kubsa + 25% Sofumer	69.2	46.3	1.1	8.9	63.3	36.4
Sofumer pure stand	55.8	42.5	-	-	47.9	29.6
Mada Walabu pure stand	17.5	10.5	-	-	13.8	5.0
25% K6295-4A + 75% Mada Walabu	24.6	16.3	55.3	57.0	41.8	13.5
50% Mada Walabu + 50% K6295-4A	37.3	29.2	32.2	23.0	25.4	21.8
75% K6295-4A + 25% Mada Walabu	51.7	31.3	6.0	17.4	20.1	25.3
K6295-4A pure stand	55.0	37.9	-	-	48.9	22.5
25% Mitikie + 75% K6295-4A	58.3	40.7	24.5	10.0	52.9	29.7
50% Mitikie + 50% K6295-4A	75.8	37.9	4.4	16.1	59.9	22.1
75% Mitikie + 25% K6295-4A	78.3	42.0	1.3	6.2	61.3	21.7
Mitikie pure stand	79.3	45.2	-	-	59.7	24.7
SE	4.08	7.08	-	-	2.0	1.4
5% LSD	11.65	20.21	-	-	10.0	12.8

SE=Standard error, LSD = Least Significant Difference

varieties of spring barely, Wolfe [15] reported reduction of up to 80% in powdery mildew infection compared with the mean disease levels of the components grown as pure stand. Browning and Frey [16] and Wolfe *et al.* [7] similarly reported that host mixtures restrict the spread of diseases when components of the mixtures differ in their susceptibility to the pathogen. In the present study, overall stripe rust reductions of up to 26% depending on the variations in mixture proportions and test environments. Stripe rust reductions in the range of 13-64% (average 34%) were noted when Wabe and Kubsa (agronomically well adapted but susceptible to stripe rust) mixed with Dure. Such widely adapted and agronomically desired cultivars but subjected to severe losses due to their susceptible to stripe rust could better be utilized if they are grown in mixtures with other cultivars having different resistance backgrounds. Mixture efficacies for the mixtures between Kubsa-Sofumer and Mitikie and K6295-4A were generally low with few exceptions. They provided average mixture efficacy of 14 and 11%. This might be due to the lack of genetic diversity between the components in terms of their resistance to the prevailing *Puccinia striiformis* populations. With few exceptions, the component cultivars used to construct the mixture showed differential reaction to the existing populations the stripe rust pathogen. Therefore it can be concluded that, although at random, there exists appropriate functional diversity matching between the resistance genes in varieties used in the mixtures combinations and the existing pathogen population. For host mixtures to restrict the spread of diseases, the components of the mixtures must differ in their susceptibility to the pathogen [11,16,17].

AUDPC Values and Disease Progression: Stripe rust progress curves and AUDPC values calculated from rust severity recorded at different time intervals demonstrated that the rate of stripe rust development in mixtures was significantly slower than the pure stand of the component varieties used in mixtures treatments formulations (Tables 1 and 2). Wabe-Dure and Kubsa-Dure mixture treatments in 1:1 proportion reduced AUDPC value as low as 45 and 44% at Sinana, 17 and 20% at Hisu and 19 and 21% at Selqa as compared the AUDPC values of 67 and

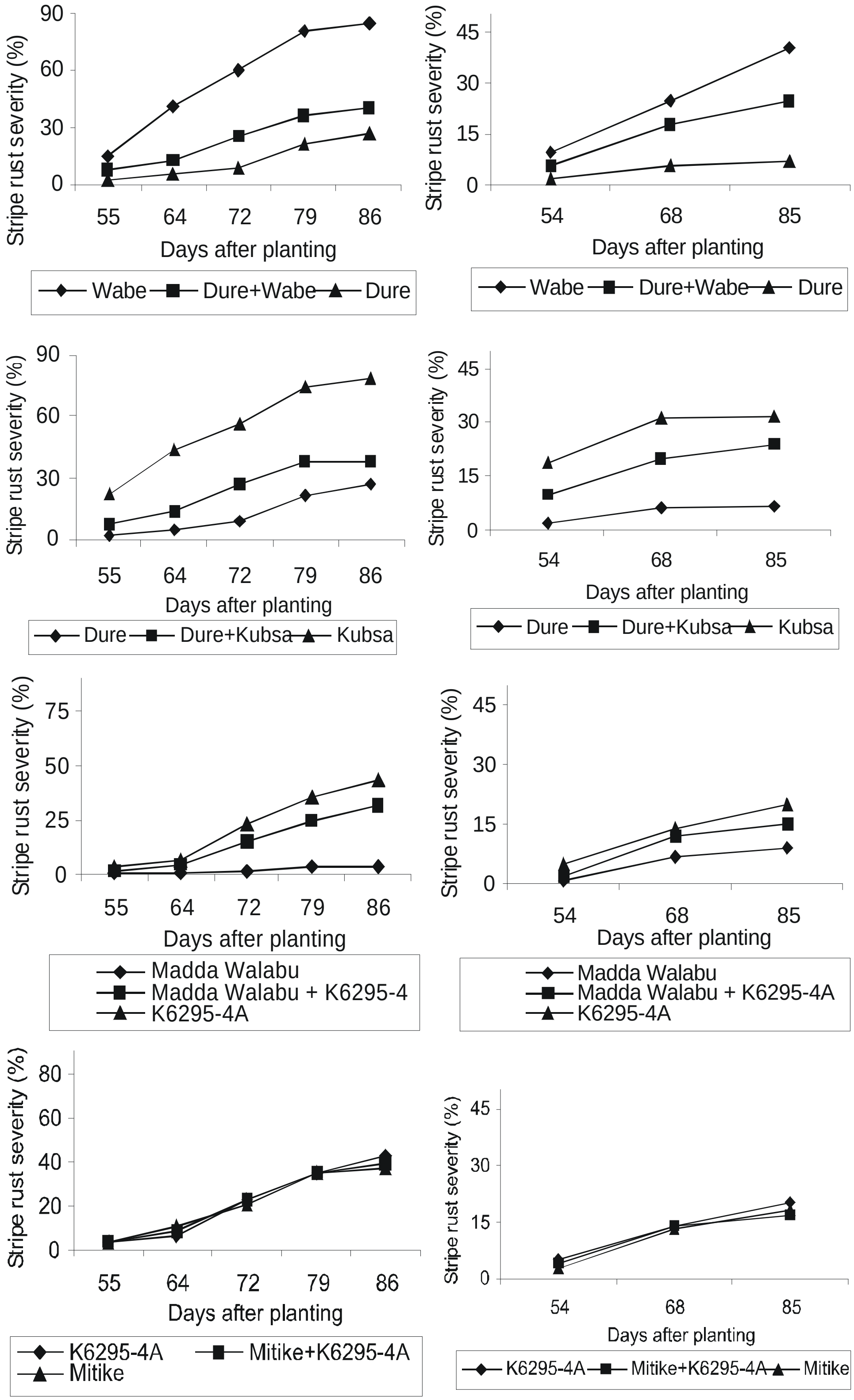

Fig. 1: Effect of variety mixtures at equal proportions of the components on the progression of stripe rust severity as compared to the pure stands of each cultivar at Sinana (left) and Hisu (right), 2004 main season

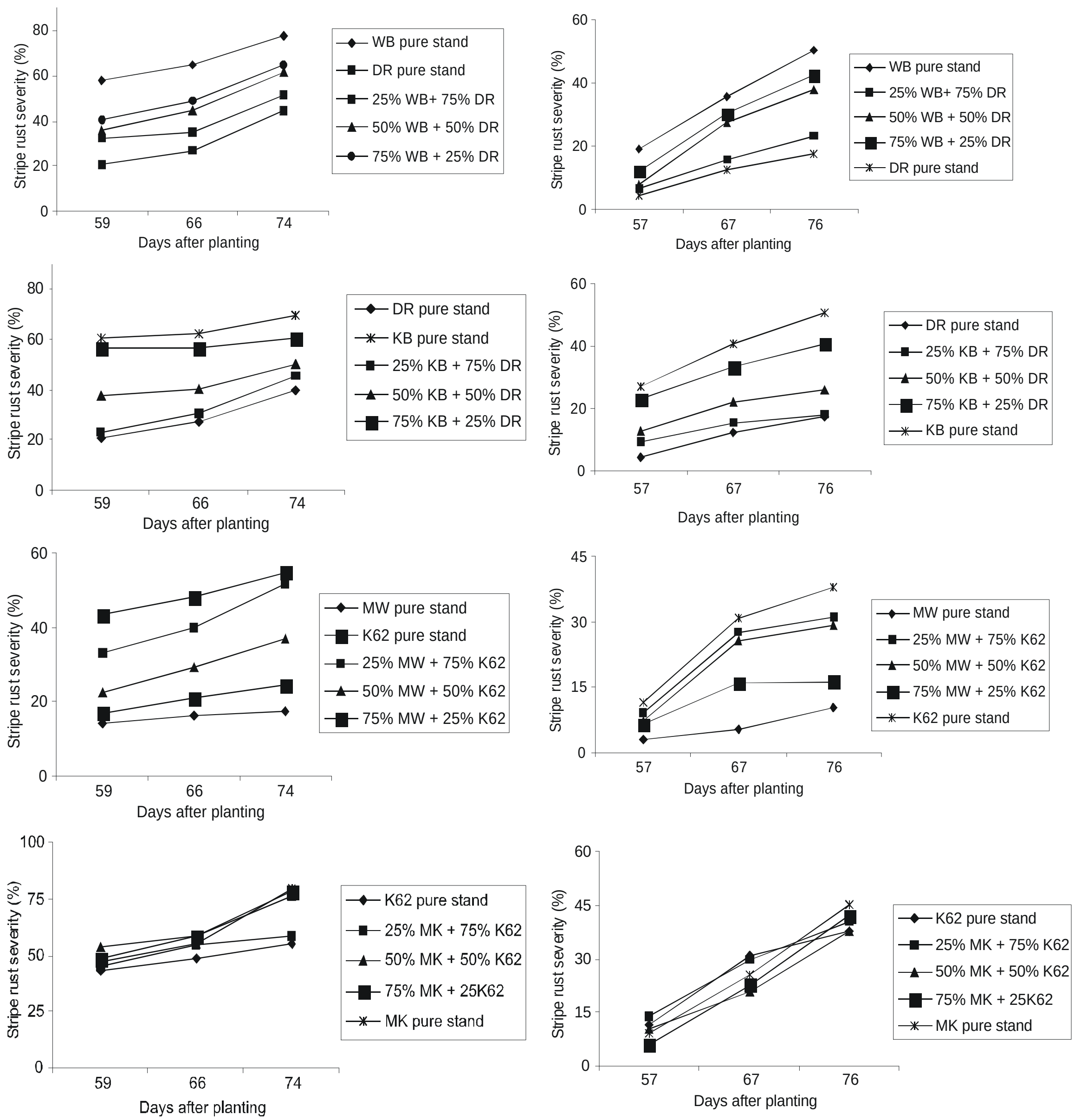

Fig. 2: Effect of cultivar mixtures at different proportions of the components on the progression of stripe rust severity as compared to the pure stands of each cultivar at Sinana (left) and Selqa (right), 2005 main season. WB = Wabe, DR = Dure, KB = Kubsa, MW = Madda Walabu, K62 = K6295-4A, MK = Mitike

63% recorded in pure stands of the susceptible components, Wabe and Kubsa, respectively, while maximum AUDPC value in pure stand of Dure was 28%. In Wabe-Dure mixtures, AUDPC values were 38 and 47% in 1:3 and 3:1 proportions, respectively, while it was 30 and 54 in the mixture of Kubsa-Dure in the respective mixture proportions at the same location. Maximum AUDPC values in Madawalabu - K6295-4A mixture treatment in equal was only 25%, while it was as high as 49% in pure stands of K6295-4A. Lannou *et al.* [9] and Trenbath [18] also reported that stripe rust progression was slower in mixtures of cultivars with different genetic background to stripe rust races.

Effect on Grain Yield: Grain yield obtained from mixtures in various proportions was mostly between the yield of the susceptible and resistant component in their pure stands (Table 3). In thirteen of the twenty mixtures

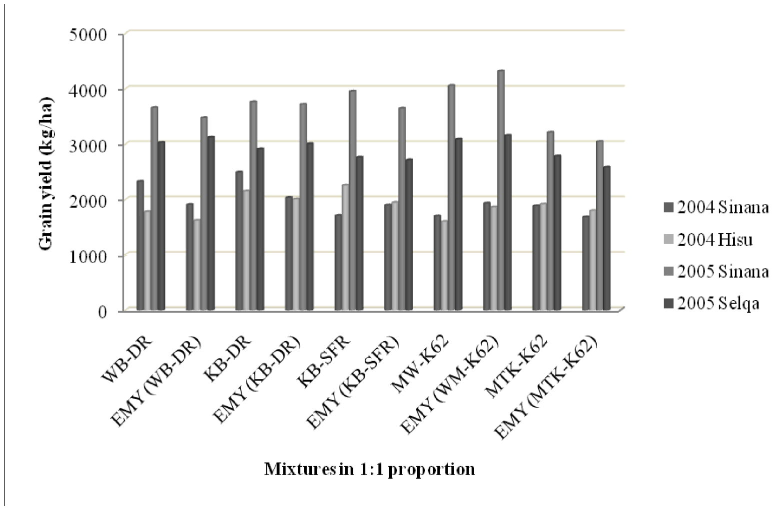

Fig. 3: Comparison of grain yield obtained from mixtures in 1:1 proportions with the expected mixtures yield from the respective component cultivars. Note: *WB-DR = mixture between cultivars Wabe and Dure, EMY (WB-DR) = Expected mixture yield for cultivars Wabe and Dure, KB-DR = mixture between cultivar Kubsa and Dure, EMY (KB-DR) = expected mixture yield for Kubsa and Dure, KB-SFR = mixture between cultivar Kubsa and Sofumer, EMY (KB-SFR) = expected mixture yield for Kubsa and and Sofumer, MW-K62 = mixture between Mada Walabu and K6295-4A, EMY (MW-K62) = expected mixture yield for Mada Walabu and K6295-4A, MTK-K62 = mixture between cultivars Mitikie and K6295-4A, EMY (MTK-K62) = expected mixture yield for Mitikie and K6295-4A.*

Table 3: Grain yield of bread wheat varieties in different proportions of cultivar mixtures and pure stand of the components at Sinana, Hisu and Selqa during the main season of 2004 and 2005

	2004		2005	
Treatments	Sinana	Hisu	Sinana	Selqa
Wabe pure stand	1138	1065.3	2890.7	2936.8
25% Wabe+ 75% Dure	-	-	3800.6	3242.5
50% Wabe + 50% Dure	2327	1777.3	3658.5	3029.4
75% Wabe + 25% Dure	-	-	3544.5	2975.6
DR pure stand	2679	2180	4058.4	3308.5
25% Kubsa + 75% Dure	-	-	3790.3	3064.5
50% Kubsa + 50% Dure	2495	2150	3761.4	2910.3
75% Kubsa + 25% Dure	-	-	3483.9	2758.5
Kubsa pure stand	1390	1831	3376.4	2703.3
25% Kubsa + 75% Sofumer	-	-	4120.2	3433.1
50% Kubsa + 50% Sofumer	1711	2255	3952.9	2764.5
75% Kubsa + 25% Sofumer	-		3588.0	2633.5
Sofumer pure stand	2407	2057.7	3917.1	2723.8
Mada Walabu pure stand	2370	1974	5610.7	3785.4
25% K6295-4A + 75% Mada Walabu	-	-	4527.2	3339.5
50% Mada Walabu + 50% K6295-4A	1702	1601.3	4058.5	3088.9
75% K6295-4A + 25% Mada Walabu	-	-	4098.8	3049.5
K6295-4A pure stand	1495	1748.3	3026.4	2531.2
25% Mitikie + 75% K6295-4A	-	-	3361.5	2773.7
50% Mitikie + 50% K6295-4A	1834	1916	3138.1	2704.8
75% Mitikie + 25% K6295-4A	-	-	3154.8	2677.8
Mitikie pure stand	1870	1848.7	3069.1	2635.3
SE	152.0	149.2	167.5	315.3
5% LSD	445.7	437.5	478.1	NS

(five mixtures in four environments) in 1:1 proportions, the grain yield obtained was higher than the expected mixture yield (mean of the two component cultivars in pure stands) (Figure 3). Those treatments provided average yield advantage of 8.6% over the expected mixture yield. In Mada Walabu-K6295-4A mixture treatment in 1:1 proportion, yield was lower than the expected mixture yield but higher than the low yielding component at three environments and lower than the low yielding at one environment, i.e. at Hisu in 2004 with a yield penalty of about 8.0% compared to the pure stand of the low yielding component (K6295-4A). Grain yield obtained from the mixture between Sofumer-Kubsa and Mitikie-K6295-4A mixture were greater than best yielding component in its pure stand at three and four environments, respectively. In all the cases of the 3:1 mixtures proportions of the resistant: susceptible cultivars, yield was greater than the expected mixture yield. In the reverse proportion (i.e. 1:3 mixtures of resistant: susceptible proportions), only three of the ten mixtures (Sofumer-Kubsa and K6295-4A-Mitikie and Wabe-Dure) could provide yield greater than the mean of the components in their pure stands.

Previous studies concluded that a useful mixture must provide yield benefit as well as disease control. According to Mundet [19] mixtures not only reduced stripe rust epidemics but also provided better yield advantage than their pure stand of the mixtures components. He reported that yield increment of 1-5% could be often provided by cultivar mixtures in the absence of substantial disease whereas; the increment is higher with higher disease development. In this study, 65% of the mixture treatments in 1:1 proportions provided yield greater than the expected mixture yield with average yield advantage of about 8.6% over the mean of the components in their pure stand. In two cases (for mixture between Kubsa-Sofumer and K6295-4A-Mitikie), mixture yielded greater than the highest yielding component. Actual yield from the mixture between Mada Walabu and K6295-4A was generally lower than the expected mixture yield most notably in 1:1 and 1:3 mixture proportions. This is could be attributed to the difference in balance of competition between the two cultivars, as there existed wide difference between the pure stand yield of Mada Walabu and K6295-4A. Pradhanang and Sthapit [12] noted that actual mixture yield and expected mixture yield could be affected by balance of competition between mixture components. The difference between actual mixture yield and expected mean yield is considerably lower and comparable when the component cultivars yield widely differed and comparable, respectively.

This study has demonstrated that cultivar mixture can provide significant level of disease restriction and improve yield if components are matched appropriately. Hence it should be considered as one of the options for stripe rust management. In areas like the Ethiopian highlands where there exists frequent epidemics and evolution new races of cereal rusts and the subsistent farmers are unable to use fungicides, the use of genetically diversified host systems through cultivar mixture is vital. It is however essential to note that randomly chosen mixture components may not necessarily provide adequate disease control. Tables of cultivars with relevant diversity to the prevailing population of the pathogen and similarity in their morphological and agronomic character should be presented to growers.

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