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STATUS OF WHITE RUST (*ALBUGO CANDIDA* (PERS. EX. HOOK) O. KUNTZE) INTENSITY AND REACTIONS OF ETHIOPIAN MUSTARD GENOTYPES FOR THEIR RESISTANCE IN WEST SHEWA, ETHIOPIA.

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ABSTRACT

Ethiopian mustard (*Brassica carinata* A. Braun) is among the oldest oil crops widely cultivated in Ethiopia. White rust (*Albugo candida* (Pers. ex. Hook) O. Kuntze) is one of the important major diseases of Ethiopian mustard in the highland areas of West Shewa, Ethiopia which is responsible for reducing the yield losses due to white rust on Ethiopian mustards estimated to be 8 – 60 %. Therefore, the aim of this study was carried out to assess the intensity of white rust and the reactions of Ethiopian mustard genotypes to white rust for their host resistance in West Shewa, during the main cropping season of 2021, in four districts viz. Dendi, Welmera, Jeldu, and Dire Incini of West Shewa zone, Ethiopia. The incidence and severity was assessed and recorded once during the survey on each farmer's field and the suspected disease symptoms of white rust on Ethiopian mustard. The incidence of white rust varied from 40.8-100% and the severity index ranged from 4–50.13%. The results were revealed that the white rust was assessed in all the study fields in Dendi district, with prevalence of 100% and in Welmera, Jeldu, and Dire Incini districts, with a prevalence of 93.3, 86.6, and 80%, respectively. The white rust intensity among different agronomic practices, at different growth stages, cropping systems and the effects of weeding and plowing frequency showed the highest white rust incidence and severity was recorded in true leaves and at pod growth stages in which both were not significantly different from each other but significantly different from cotyledons. The maximum level of white rust incidence and severity was recorded at a high altitude level of 2413 m.a.s.l. in the study areas. The field experiment was conducted at Holleta Agricultural Research Centre, to evaluate 20 Ethiopian mustard genotypes for host resistance against white rust during the main cropping season of 2021/22. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications of each treatment. The results under field conditions revealed that among 20 genotypes tested, two genotypes (S-67xHoletta-1-9/2/18/2/37/4/1 and Yellow dodola) were highly resistant, followed by 3 genotypes (Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1, S-67xHoletta-1-9/2/18/2/24/2, and S-67xHoletta-1-9/2/18/2/37/4) were resistant, five genotypes (S-67xHoletta-1-9/2/18/2/45/3, Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3, Y.D.xBAR1030/79-436/2001/6/2/11/1/18/2, S-67xHoletta-1-9/2/18/2/37/3 and S-67xHoletta-1-9/2/18/2/37/3) were moderately resistant, while the other 10 genotypes were susceptible. The least AUDPC value was recorded from S-67xHoletta-1-7/1/13/2/26/2. Overall, the present study concluded that S-67xHoletta-1-9/2/18/2/37/4/1 and Yellow dodola were highly resistant, which can be easily adopted by the farmers to control white rust in their cultivated fields. Therefore, the current study suggests that the prevalence of the pathogen and the reactions of tested lines that rescuer further evaluation of resistance across the locations.

Keywords: Ethiopian mustard, White rust, *Albugo candida*, Intensity, Genotypes, Host Resistance.

INTRODUCTION

Ethiopian mustard (*Brassica carinata* A. Braun) is an amphidiploid species that belongs to the family *Brassicaceae* or cruciferous, which is originated from the highlands of Ethiopia and adjoining regions of East African and Mediterranean coast through inter-specific hybridization between *Brassica nigra* L. Koch and *Brassica oleracea* L. with genomes of BBCC, 2*n* = 34 (Getinet *et al.,* 1994). It is locally known as "Raafuu" in Oromic language, "Gomenzer" ("Yehabesha Gomen") in Amarainya and "Hamli Adri" in Tigrigna and commonly known as Abyssinian cabbage, Abyssinian mustard, Ethiopian kale, Ethiopian mustard, Ethiopian rape and Mustard collard (Sharma *et al.,* 2022).

Ethiopia is the center of *B. carinata* genetic diversity, and its cultivation began there around 4000 years ago. Utilization of the available germplasm of *B. carinata* for different breeding purposes requires information on genetic diversity. Production of *B. carinata* for its seed is important only in Ethiopia. It is utilized as leaves as vegetables and oil crops. Farmers in the country's highlands cultivate it as a green vegetable in their gardens and it can be used for multipurpose, such as increasing farmers' income and creating environmentally sustainable business. It is an oilseed crop grown extensively in the highlands of Ethiopia, but as a leafy vegetable, it is often grown in East and Southern Africa, less so in West and Central Africa. The leaves of Ethiopian mustard plants are high in vitamin C and K, beta-carotene, and cancer-fighting antioxidants, as well as being low in bitterness (Getinet *et al.*, 1994).

Ethiopian mustard is annually grown on about 45,200 ha of land with a total harvest of about 74,700 tons (CSA 2019). It is grown in a temperate climate at higher elevations and the optimum temperature required for growth and development is 18–25 °C with low humidity.

Soil pH of 6.0-7.5 is ideal for proper growth and development (Chattopadhya *et al*., 2011). Soil pH less than 5.0 is best, but pH 9.0 is detrimental to those *Brassicas*. It is also a drought and heat-tolerant oilseed crop (Hagos *et al*., 2020). Ethiopian mustard has spread throughout most semi-arid climate regions, including Europe and South Asia, and is now being employed as an alternative energy crop in marginal lands. *B. carinata* has deep roots, a low canopy temperature, thick waxy leaves and a high temperature tolerance, making it ideal for moisture-stressed environments (Singh, 2003). Ethiopian mustard can grow best at mid to high altitudes (2000–2600 m.a.s.l.) on more fertile, well-drained soil, which is generally near to the farm house in Ethiopia. The main growing regions for Ethiopian mustard are in Arsi, Bale, Gonder, Gojam, Wello, Shewa, Sidamo, and Wellega. For the past five years, Ethiopia produced between 550,000 and 750,000 quintals in areas with a 30,000 to 45,000 ha range (CSA, 2019). It is grown in 44, 464.46 ha areas in Ethiopia, with a yield and productivity of 4.3 million quintals and 97.4 quintals per ha, respectively (Tiwari *et al*., 2021). Ethiopian mustard was grown on 14,661.2 ha in the Oromiya region, with a yield and productivity of 90.8 quintals ha and 1.3 million quintals, respectively. Oromia's 90.8 quintals ha output, compared to the country's 97.4 quintal ha is attributable to a variety of problems limiting the cultivation of this crop (CSA, 2019).

Many diseases attack *Brassica spp.* in Ethiopia, such as white rust (*Albugo candida),* leaf and pod spot (*Alternaria brassicae*), leaf spot (*Alternaria tenuissima),* powdery mildew (*Oidium spp.*) and downy mildew (*Peronospora parasitica)* (Saharan and Verma, 1992). White rust (WR) or Stag heads are caused by several species of the fungus, *Albugo candida*, which is the only genus in the family Albuginaceae in the Order Peronosporales of Class Oomycetes. It is an obligate pathogen and has a relatively smaller genome as compared to other Oomycetes (Links *et al*., 2011). More than 30 diseases are known to occur on *Brassica* crops, including Ethiopian mustard and rapeseed mustard in India (Saharan *et al*., 1992; Choi *et al.,* 2008). Ethiopian mustard is seriously affected by fungal diseases; the most common of which is white rust, According to reports, *Albugo candida* is to blame for this, which causes extremely large yield losses that become apparent when the first signs of white rust are noticed. The first signs of disease can be observed on cotyledons as early as the beginning of December. On the leaf side of the cotyledons and on the main leaves, the WR disease first appears as white to cream-yellow raised zoosporangial pustules of varying shape and size (Gupta & Saharan, 2002). Except for the roots, all plant parts are impacted by the fungus, which appears in both vegetative (local) and reproductive (systemic) tissues (Shek *et al.,* 2017).

However, the spread, intensity, and yield loss caused by white rust have yet to be as well determined. In India, Bisht *et al.* (2018) recognized the spread, intensity, and yield loss of white rust; but there had been no study of the disease's distribution or resistance lines in Ethiopia. Although, many chemicals as well as cultural measures (Barbetti, 1988; 1988a; 1988b) have been suggested for controlling of this disease, but genetic resistance is the most efficient and cost-effective approach of protecting mustard plants from white rust. White rust resistance has been reported to have been discovered in *B. alba, B. campbell, B. carinata* (HC-1), *juncea, and B. napus*. The identification of sources of resistance has been used to try and control WR through host resistance (Petrie 1988; Saharan *et al.*, 1988; Tiwari *et al.*, 1988). The majority of the genes that control each source of resistance that have been found so far are race-specific. Resistance breeding is currently the most effective and environmentally friendly approach to control WR.

Surveys conducted earlier revealed that white rust has been found to be one of the major diseases of Ethiopian mustard in the West Shewa zone. However, limited work has been done on the importance, distribution, and effects of the disease on the quantity and quality of the Ethiopian mustard crop, which has not been investigated in West Shewa, Ethiopia. Despite the frequent occurrence of severe epidemics of WR disease in the Ethiopian mustard growing areas of the country, there is no adequate information on the status of the pathogen distribution and disease intensity in the West Shewa zone of the Oromia region. The identification of sources of host resistance is a necessary step in using effective host resistance to combat this disease. However, the most efficient means of controlling this disease in Ethiopia is the survey and reaction of host resistant genotypes. Utilization of the available germplasm of *B. carinata* for different breeding purposes requires information on genetic diversity. Therefore, this study was undertaken to assess the status of Ethiopian mustard white rust disease intensity and the reactions of Ethiopian mustard genotypes to host resistance under field conditions in Holleta Agricultural Research Center, Western Shewa Zone, Oromia Regional State, Ethiopia to improve the production and productivity of Ethiopian mustard.

MATERIALS AND METHODS

Description of the study areas

The disease survey was conducted from seedling to maturity growth stages of the Ethiopian mustard crop in the West Shewa zone, Oromia Regional State, Ethiopia, during the main cropping season of 2021. The West Shewa zone is located at latitude 8° 17'8°57'N and longitude 37°08'-38°07'E, with elevations ranging from 1380-3300 m. a. s. l. and annual rainfall ranging from 600-1900 millimeters. The study localities have a bimodal rainfall distribution and also typical sub-humid and high altitude agro-climatic zones. The mean minimum and maximum air temperatures in the area are 17.20 °C and 24.40 °C, respectively. The field experiment was conducted at Holleta Agricultural Research Center during the 2021–22 main cropping seasons. Holleta is

33 kilometers west of Addis Ababa, at 09°02' 55.500" (N) Latitude, 038°30'06.660" (E) Longitude, with an altitude range of 2340 m. a. s. l. The average annual precipitation (mm) was 1144 mm and the average annual temperature was 6 °C. The organic C content was 1.18% and 0.16%. The soil type is Nitosol with a pH of 6.0.

Survey of white rust intensity in Ethiopian mustard

The survey was conducted in four districts, viz., Dendi, Jeldu, Welmera, and Dire Incini in West Shewa Zone, Oromia Regional State, Ethiopia during the main cropping season of 2021 (Figure 1). Purposive sampling was used to select three localities from each district: Dendi (Galessa Kota Gesher, Galessa koftu and Kebabereda), Jeldu (Sariti Denku, Chilenko and Kolugelan), Welmera (Wetabech Menjero, Markos and Burkusami Gaba Robi) and Dire Incini (Omiane, Nano gidu and Bola Germema). A simple random sampling method was applied to the sampled farm field. A total of 60 Ethiopian mustard farmers' fields were visited in the areas surveyed, where 15 fields were randomly assessed from each district. Data was collected in each sample field from five points along the two diagonals using a quadrant (4m x 4m). The plants in each quadrant were taken as the sample unit while the leaves and pods of the plants were thoroughly inspected. Five plants were randomly chosen from each of the four (4 m2) quadrants and assessed for disease incidence and severity using data from their farmer's field, formed as replication.

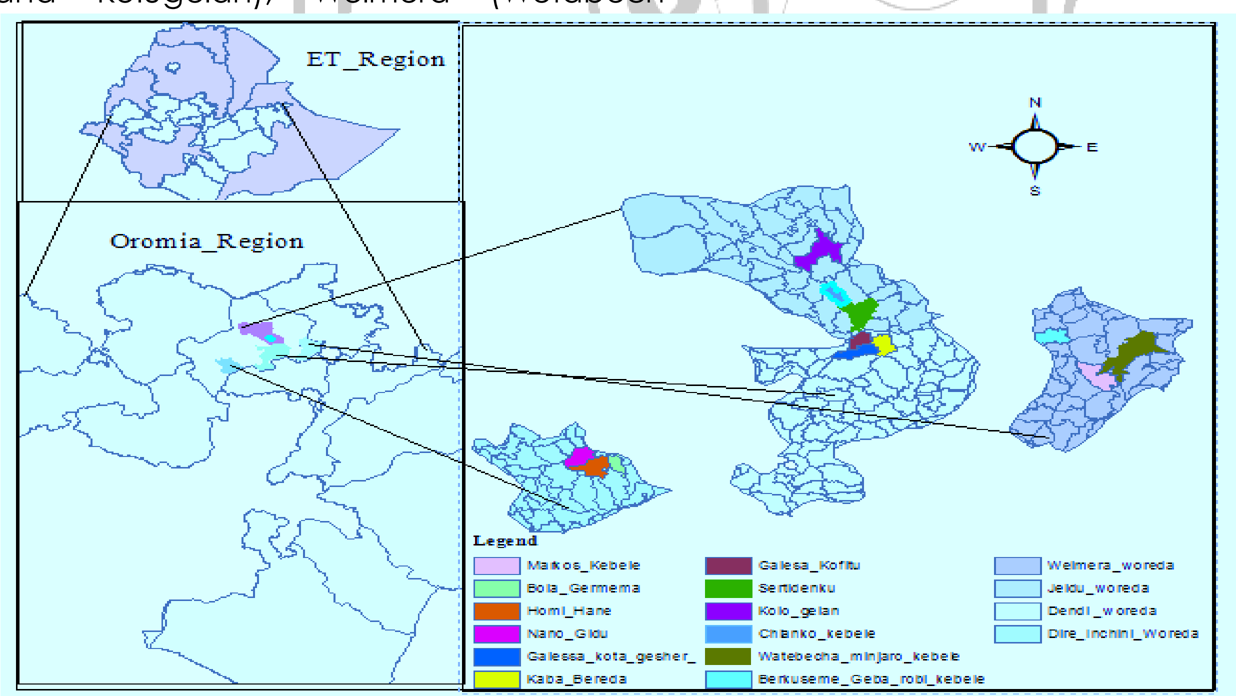


Figure 1: Map of the study areas

$$\text{Disease Incidence}(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} * 100$$

Disease parameters from each farmer's field, taking a plant every two meters until a total of 25 plants were collected. Disease prevalence, incidence, and the severity was assessed and recorded once during the survey on each farmer's field (August 1 to 20 days, 2021)

Disease prevalence (%)

The disease's prevalence was calculated by dividing the number of fields affected by the total number of fields assessed and expressing the result.

$$\text{Prevalence }(\%) = \frac{\text{No of field affected the disease}}{\text{Total number of field assessed}} * 100$$

Disease incidence (%)

The incidence of white rust was calculated by using the number of infected plants and expressed as a percentage of the total number of plants assessed.

Disease severity

Disease severity was recorded by visually estimating the percentage of leaf area diseased from twenty-five (25) random plants taken and six leaves per plant in the farmer's field. Each point was selected to record white rust severity. The severity was assessed and recorded once during the survey on each farmer's field (August 1 to 20 days, 2021) Suspected disease symptoms white rust Ethiopian mustard. The data on disease severity was recorded using a 0–9 rating scale according to Pound and Williams' (1963) modified by Singh *et al.* (2002) (Table 1). Lastly, each severity scale was converted into the Percentage Severity Index (PSI) for analysis.

Table 1. White rust severity and Percent Index Severity scoring scale

Rating Scale	Description
0	No symptoms or sign of *A. candida* infection
1	Pinpoint necrotic flecks at inoculation site, no sporulation
2	Larger necrotic flecks at inoculation site, no sporulation
3	Sparse sporulation, upto 5 % of surface covered with pustules
4	6–10 % of leaf area covered with pustule
5	11–20 % of leaf area covered with pustules
6	21–30 % of leaf area covered with pustules
7	31–50 % of leaf area covered with pustules
8	51–75 % of leaf area covered with pustules
9	>75 % of leaf area covered with pustules

Percent severity index (PSI)

The disease severity index was calculated using the following formulae: (Singh *et al.*, 2002).

$$\text{Percent severity index} = \frac{sum\ of\ numerical\ ratings}{number\ of\ plants * maximum\ score\ grade} * 100$$

Area under Disease Progress Curve (AUDPC).

AUDPC was computed from the PSI data recorded at each date of assessment as described by Campbell and Madden (1990). AUDPC was expressed in percent-days because the severity (x) is expressed in percent and time (t) in days. The area under Disease Progress Curve was calculated using the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where, Yi= disease severity on the ith date, Y (i+1) = disease severity on the i+1th date, n = number of dates.

Pathogenecity test

The pathogenicity of conidial isolates of *Albugo candida* was confirmed by Koch's postulates by the method of Parisi and Lespinasse (1996) under laboratory conditions at Holotta Agricultural Research Center, Plant Pathology Lab. The pathogenicity test of the identified pathogen, *Albugo candida* was tested on diseased free detached leaves (Thin *et al.,* 2008).

Detached leaf technique

Preparation of spore suspension

To prepare a spore suspension for white rust conidia, local sori were placed in 3 ml of sterile distilled water and rapidly shaken for 3 minutes (Onyeani *et al.,* 2012). The resulting suspension was run through two layers of cheese cloth for filtration. A hemocytometer was used to regulate the spore suspension's concentration to 1x10^6 spores or conidia/ml. The pathogenicity test was carried out in a laboratory experiment using diseased free-detachment leaves from Ethiopian mustard that were procured from the Holotta Agricultural Research Center in the greenhouse. These leaves were then washed with autoclaved water, surface sterilized with 70% ethanol, and then placed on five larger plastic Petri dishes with moist tissue paper lined on the inside. *Brassica carinata* seedlings that were 12-to 14-day-old were separated from their leaves and cultured on a medium containing 0.5 ppm of benzyl adenine and 0.8% agar in 25 ml of autoclaved etri dishes. Usually, within 15 minutes of detachment, leaves were placed in the dishes with their lower surface on the medium. *A. candida* sporangia-derived zoospore suspension (100,000/ml) was used to drop-inoculate leaves. A pathogenicity test of the pathogen was required to determine whether the white rust disease was actually caused by a fungus or not (Parisi and Lespinasse, 1996). Observations and comparisons between the symptoms of the lesions that developed on the inoculated leaves and those described for naturally infected field leaves were made. Beginning seven days following the inoculation, periodic inspections were made for the emergence of symptoms on the leaves, and these findings were later confirmed.

Evaluation of Ethiopian mustard genotypes for host resistance against white rust.

Description of genotypes

A field experiment was conducted during the 2021–2022 main cropping season at Holleta Agricultural Research Centre experimental station (Figure 2). 20 different Ethiopian mustard genotypes were screened for WR resistance seeds of Ethiopian mustard genotypes, including four improved varieties and 16 breeding lines (advanced varieties) with one local cultivar, for a total of twenty genotypes evaluated (Table 2).

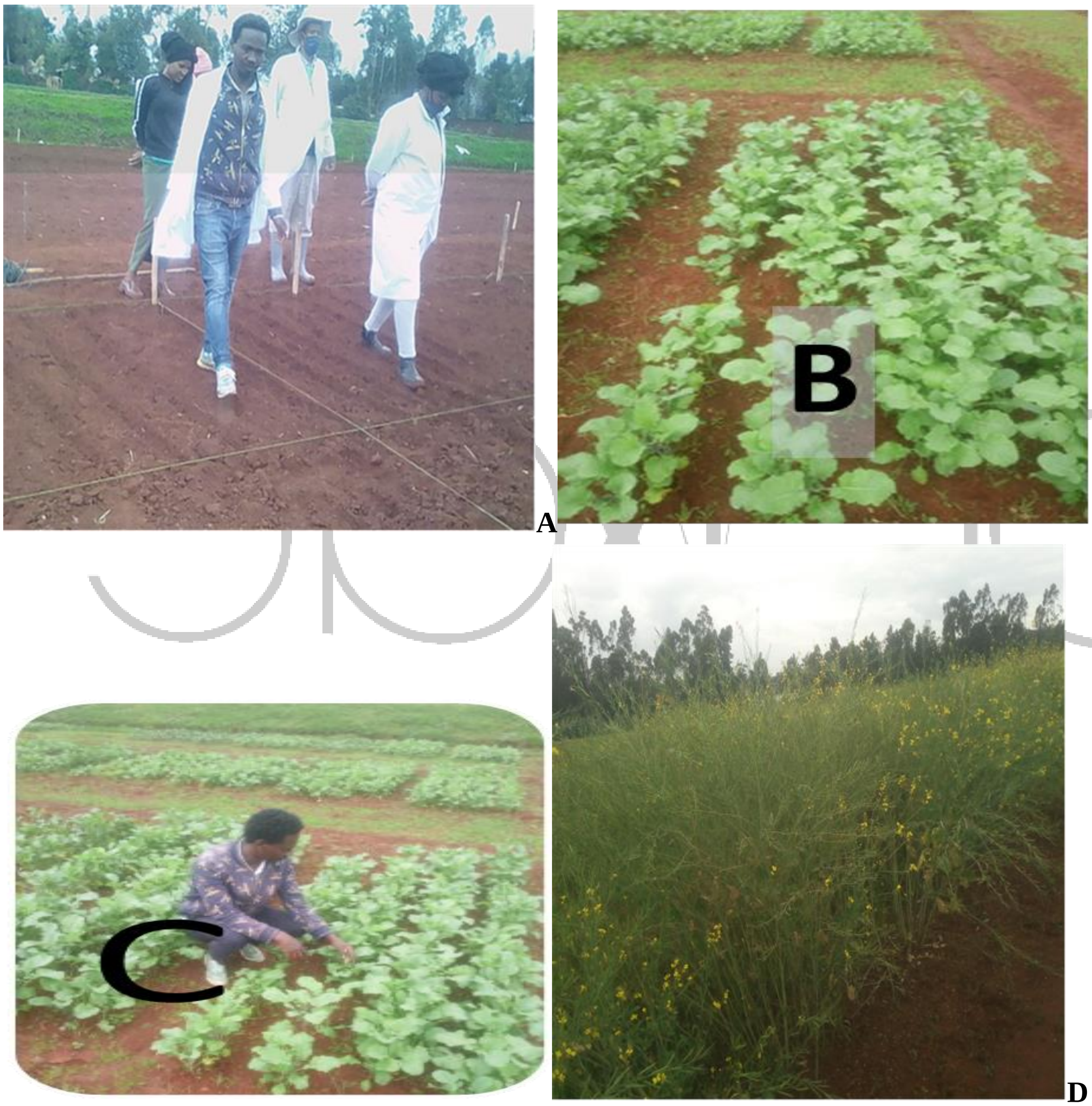


Figure 2: A. Experimental Layout. B, C & D. Reaction of Ethiopian mustard genotypes against white rust at Holleta Agricultural Research Center Experimental field.

Table 2: Ethiopian mustard genotypes evaluated

S. No	Genotypes	Status of genotypes
1	S-67xY.D.3/1/5/1/9/4	Breeding line
2	S-67xHoletta-1-9/2/18/2/37/4	Breeding line
3	S-67xHoletta-1-7/1/13/2/26/2	Breeding line
4	S-67xY.D.2/2/4/1/7/3	Breeding line
5	Y.D.xBAR-1030/79-436/2001/6/1/10/1/15/3	Breeding line
6	S-67xHoletta-1-5/2/10/2/20/4	Breeding line
7	S-67xHoletta-1-9/2/18/2/37/3	Breeding line
8	Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3	Breeding line
9	S-67xHoletta-1-9/2/18/2/45/3	Breeding line
10	S-67x34477 Pakistan 5/2/9/2/14/3	Breeding line
11	S-67xHoletta-1-6/2/12/2/24/2	Breeding line
12	Y.D.xBAR-1030/79-328/2001/8/2/13/1/22/4	Breeding line
13	Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/2	Breeding line
14	S-67xHoletta-1-9/2/18/2/37/4	Breeding line
15	Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1	Breeding line
16	Local cultivar	Local, Susceptible
17	Yellow dodola	Released variety,Resistant
18	S-67	Released variety
19	Tesfa	Released variety
20	Derash	Released variety

Experimental design and Management

The field experiment was laid out with 20 genotypes treatments in a Randomized Complete Block Design (RCBD) with three replications. The seeds of 20 cultivars were surface sterilized by immersing them for 10 minutes in a 0.6% sodium hypochlorite solution and then vigorously rinsing them with distilled water. After drying for 24 h on filter paper, the seed rate of 20 genotypes was separately per hectare. 5 kg were planted in three rows using 0.3m space between plants and 0.6m space between plots for the experiment. The area of each plot was 1.2 x 2 m to maintain an optimal plant population, and the surplus plants were thinned out at 15 days after sowing. The plots were fertilized at a rate of 60:40 (N: P), which was used in three rows in each plot.

On the plot, an experiment between two boarders of rows was conducted. Ten plants were randomly selected in each plot using a simple random sampling technique. Furthermore, the natural visible conidia spore of white rust on mustard cultivar crop growth stages was at 50 DAS in our experiment field under the main cropping conditions we considered in those authors (Shrestha *et al.,* 2021). Ethiopian mustard was used as a standard check yellow doddola and control, respectively.

White rust disease assessment

Disease assessment was started as soon as disease occurred on 50 DAS based on the reaction of the differential cultivars and promising mustard cultivars (Sahara *et al.,* 1988). The incidence of white rust was calculated by using the number of infected plants and expressed as a percentage of the total number of plants assessed (Bisht *et al.*, 2018):

$$\text{Disease Incidence}(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} * 100$$

Disease severity: The severity of the disease was examined and recorded as the percentage of plant parts (tissue) affected (percentage of white rust infection of the plant). At true leaves, different mustard genotypes were evaluated. The white rust disease first appeared on mustard leaves and pods (stag head formation) between 36 and 131 days after sowing (Chattopadhyay *et al.,* 2011). Observations for Percent Disease Severity (PDS) were recorded at ten-day intervals following the harvest of ten randomly tagged plants in each plot on the leaves of following the scale of Singh *et al.* (2002). For each assessment date, PDS on leaves from 10 tagged plants from each plot was averaged to give respective single values. The observations on disease severity and disease reaction were made using a 0-9 grading scale (Table 3) based on all leaves of 10 randomly selected plants from each genotype at the highest disease pressure, i.e., at 75 DAS (Bisht *et al.*, 2016).

Table 3: Rating (0-9) scale for measuring disease severity and disease reaction

Rating score	Leaf area covered (%)	Disease reaction
0	No symptoms	Immune
1	< 5	Highly resistant
3	6-10	Resistant
5	11-25	Moderately Resistant
7	26-50	Susceptible
9	<50	Highly

Source: (Bisht *et al.*, 2016).

Area under Disease Progress Curve (AUDPC).

AUDPC was computed from the PSI data recorded at each date of assessment as described by Campbell and Madden (1990). AUDPC was expressed in percent-days because the severity (x) is expressed in percent and time (t) in days. The area under Disease Progress Curve was calculated using the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where, Yi= disease severity on the i^{th} date, Y (i+1) = disease severity on the i+1th date, n = number of dates.

Relative Yield loss of Ethiopian mustard genotypes against white rust

Ethiopian mustard yield was different among genotypes compared to all plots. It was measured as a percentage of yield reduction (kg) and used to show a difference in yield with the same management on those plots of Ethiopian mustard genotypes, to comparison the effect of white rust on Ethiopian mustard yield (Saharan,1993).

Data Analysis

The data of field experiments were subjected to Analysis of Variance (ANOVA) as Randomized Complete Block Design (RCBD) using SAS version 9.3 statistical software, which was used to examine the data (Stokes *et al*., 2012). After a Kolmogorov-Smirnov analysis revealed significant differences (P>0.05) and a non-normal distribution, the survey values were transformed using ARCSINE. With the exception of farmers' fields, which were treated as a random effect, fixed factors were structured in two stages of nested design (Ababa *et al*., 2021; Binyam *et al.*, 2014). In the two levels of nested design, the fields were nested as replications under farmers; farmers were nested under kebeles; and kebeles were nested under districts. Mean separation was done using the Least Significant Difference (LSD) at a 5% probability level. The associations between white rust intensity and agronomic practices, altitude, and crop growth stages were investigated using Pearson correlation, and the magnitudes of white rust intensity were predicted using simple regressions. The Duncan's multiple range tests were used to analyze the mean comparison of the genotypes.

Results and Discussion

Ethiopian mustard white rust intensity in West Shewa

During the survey, the incidence of white rust on Ethiopian mustard was not significantly variable in four districts of West Shewa. Figure 3 showed white rust symptoms on true leaves and pods. The disease was recorded in all study fields of the Dendi district, with a prevalence of 100 %, whereas in Welmera, Jeldu, and Dire Incini districts, with prevalence's of 93.3, 86.7, and 80.0%, respectively (Table 4). The highest occurrence of white rust prevalence (100%) was found at 2413 m.a.s.l in the highlands of the study areas. Out of 12 kebeles tested, the white rust prevalence was high (100%) at Wetabech Menjero, Markos, Galessa Kota Gesher, Galessa koftu, Kebabereda, and Kolugelan kebeles. As shown in Table 4, the maximum disease incidence (66.4%) and severity (21.6%) were recorded in Dendi district. However, the minimum incidence (45.6%) and severity (10.0%) were recorded in the Dire Incini district. indicating that the result of white rust was highly expanded and severely might have reduced the quantity and quality of Ethiopian mustard crops in West Shewa districts. Overall, the mean incidence and severity of white rust disease in Dendi (66.4% and 21.6%), Welmera (65.6% and 21.0%), Jeldu (57.3 % and 19.0%) and Dire Incini (45.6 % and 10.0%) districts, respectively (Figure 4). The first survey report suggested that white rust wasn't recorded in all districts of West Shewa, primarily in the form of stage and staghead, but *Brassica carinata* was shown to be more sensitive to these diseases than *Brassica napus* among the species (Stewart and Yiroou, 1967). The results of the present study conform to those reported earlier by Shek *et al.*, (2017) and similarly, a group of researchers also reported earlier the white rust intensity in Indian mustards, which is in agreement with the present results (Bisht *et al.*, 1994; Gupta and Saharan, 2002; Bisht *et al.*, 2018).

The incidence and severity of white rust on Ethiopian mustard differed greatly among the Kebeles. The maximum incidence (100%) and severity (50.13%) were recorded at Markos kebele in the Welmera district, whereas the minimum incidence (34.4%) and severity (4.0%) were recorded at Burkusami Gaba Robi kebele, similarly in the Welmera district. The mean comparison showed that the severity at Markos Kebele differed significantly from that of Keba Bereda Kebele. The Wetabech Menjero, Omiane, Bola germema, and Burkusami Gaba Robi were not significantly different from one another, but significantly different from the previous two Kebeles. Nevertheless, the severity of white rust was the same in all seven localities (kebeles) The incidence of Markos and Burkusami Gaba Robi differed greatly, while the remaining kebeles were all the same (Table 5).

Table 4. Intensity of white rust caused by *Albugo candida* in Ethiopian mustard during the main cropping season 2021 in four districts of West Shewa zone.

Districts		Kebeles	Prevalence (%)	Incidence (%)	Severity (%)	Altitude m.a.s.l.
	Welmera		93.3	65.6	21	--
		Wetabech Menjero	80	62.4	8.71	2704
		Markos	100	100	50.134	2413
		Burkusami Gaba Robi	80	34.4	4	2604
	Dendi		100	66.4	21.6	--

		Galessa Kota Gesher	100	60.8	18.85	2998
		Galessa koftu	100	64.8	17.96	3013
		Kebabereda	100	73.6	28.09	3011
	Jeldu		86.7	57.3	19	--
		Sariti Denku	80	53.6	17.42	3029
		Chilenko	80	56	20.35	2948
		Kolugelan	100	62.4	18.49	2816
	Dire Incini		80	45.6	10	--
		Omiane	80	44.8	8.35	2479
		Nanongidu	80	51.2	12.62	2457
		Bola Germema	80	40.8	8.45	2518

Table 5: White rust severity index and incidence % in different kebeles during the main cropping season 2021 in West Shewa.

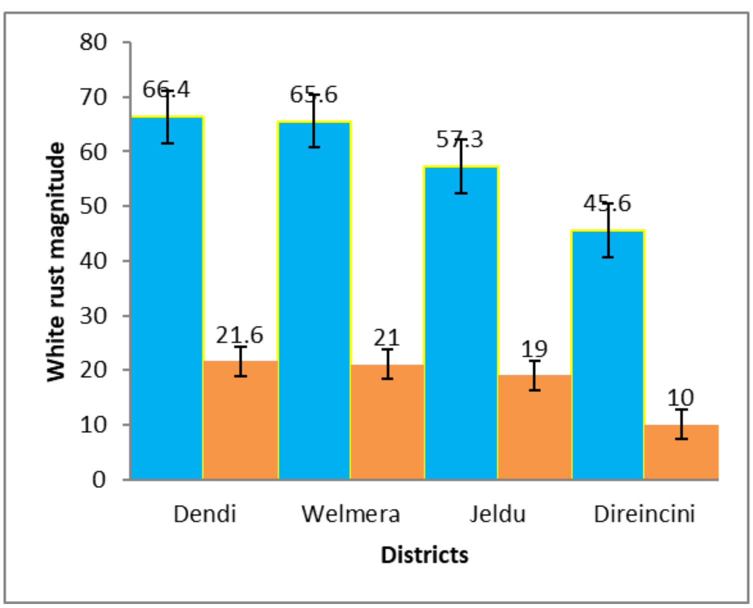
Kebeles	Incidence %	Disease severity index%
Markos	100a	50.13a
Kebabereda	73.6ab	28.1b
Galessa koftu	65ab	18bc
Wetabech Menjero	62.4ab	9c
Kolu Gelani	62.4ab	18.5bc
Galessa Kota Gesher	60.8ab	19bc
Chilenko	56ab	20.3bc
Sariti Denku	53.6ab	17.4bc
Nano Gidu	51.2ab	12.6bc

Omiane	44.8b	8.3 c
Bola Germema	40.84b	8.4c
Burkusami Gaba Robi	34.4b	4.0 c
CV (%)	39.8	47.6





Figure 3. Screening of White rust disease intensity on leaves and pods of Ethiopian mustard at cultivated fields of Dendi, Welmera, Dire Incini and Jeldu districts of West Shewa Zone.



Incidence

Severity

Figure 4. Overall mean percentages of Ethiopian mustard white rust incidence and severity during the main cropping season 2021 at four districts of West Shewa Zone.

White rust intensity among different agronomic practices

This study's results showed that all districts were affected by white rust but the disease intensity was variable across the assessed districts (Tables 4 & 5; Figure 4). Various environmental and agronomic factors like intensity and distribution of rain fall, soil type, altitude ranges, temperature, tillage system, plant growth stages, type and source of Ethiopian mustard seeds used for production, cropping systems practiced by growers, and other agronomic practices applied by farmers result in variation in the distribution and intensity of the disease across localities/districts as well as farms. Similarly, Bisht *et al.* (1994) reported that previous crop residue and tillage practices were differentially affected by the incidence and severity of Fusarium head blight.

White rust intensity at different growth stages

The white rust intensity was significantly different at different growth stages of Ethiopian mustard (Table 6). The highest incidence was at true leaf growth and pod stages, but this was not significantly different from true leaves and pod growth stages. The lowest incidence was recorded at the cotyledon growth stage and was significantly different from the two growth stages. The highest white rust severity was at true leaf and pod growth stages in which both of them weren't significantly different from them but significantly different from cotyledon (Table 6).

Table 6: White rust intensity at different growth stages of Ethiopian mustard

Growth stages	Incidence (%)	Severity (%)
Cotyledon	9.6^{b}	1.1^{a}
True leaf	63^{a}	43.3^{b}
Pod	56^{a}	17.1^{b}
LSD	24	12.4
CV (%)	36.5	29

Effect of cropping systems on white rust intensity

As nested ANOVA indicated, crop rotation affects the white rust intensity. The mean comparison showed that a different crop cultivated in the previous cropping year was affected in significantly different ways by white rust intensity. Onion crops cultivated in the previous cropping season resulted in the highest incidence of white rust and were significantly different from Faba bean crops cultivated. On the other hand, crops that were cultivated in previous cropping seasons weren't significantly different from each other. This indicates that rotation of teff with mustard, wheat with mustard, and lettuce with mustard may not affect the white rust of Ethiopian mustard (Table 7).

Table 7: Effects of cropping system on white rust intensity during 2021

Cropping system	Incidence	Severity
Onion	80[a]	31[a]
Teff	74[ab]	28[ab]
Wheat	72[ab]	26[ab]
Mustard	72[ab]	23[ab]
Inset	62[ab]	22[ab]
Barley	59[ab]	20[ab]
Lettuce	56[ab]	15[ab]
Potato	52.2[ab]	13[ab]
Maize	38[b]	7[ab]
Fababean	0[c]	0[b]
LSD (%)	37.9	24.4
CV (%)	36	77

Effects of weeding and plowing frequency on white rust intensity

The white rust incidence was significantly affected by time of hand weeding but not by plowing frequency. Then, the mean comparison indicated that the effects of two, three, and four hand weeding practices were not significantly different on white rust, but that the effect of one-time hand weeding was significantly different from the former on white rust incidence. The highest disease incidence was scored at one hand weeding frequency and the lowest disease incidence was at four hand weeding. The severity of white rust was not significantly affected statistically by four, three, two, and one hand weeding practices. But, the severity of white rust, mathematically, was 3.2 % from one to four hand weeding practices and 35% maximum from one-time hand weeding. Table 8 indicates the mean severity of white rust but not CV and LSD due to the ANOVA indicates non-significant. The white rust of Ethiopian mustard was not significantly affected by the plowing frequency. The incidence of white rust was highest at one plowing frequency, at 80% and at 35% severity. However, the effects of four, three, and two times plowing frequency were not significantly different. The effects of plowing frequency on white rust incidence and severity are listed (Table 8; Figure 5). The degree of white rust is affected by both manual weeding and plowing frequency. This study suggests that weed removal is important, as the degree of white rust was reduced dramatically in the field when manual weeding was done at two and above. This is because as the population of plants decreases as weeds are removed, the microclimate that maintains a comfortable temperature and humidity for the disease is lost (Mehta, 2019). Another reason for the decline in white rust is that mustard is able to survive the disease because nutrient competition is reduced due to good weed control. In the context of plant protection, land plowing entails exposing the disease inoculum to sunlight, breaking the disease dormancy, and creating an unfavorable environment for the diseases. According to this concept, land plowing had an impact on white rust in the current investigation. Plowing the land more than twice can help to reduce the incidence and severity of white rust.

Table 8: Effects of weeding on white rust intensity

Time of weeding	Incidence %	Severity%
Four	21 [a]	32
Two	65 [a]	20
Three	61[a]	19
One	80[b]	35
LSD	26	19.2
CV (%)	36	76.7

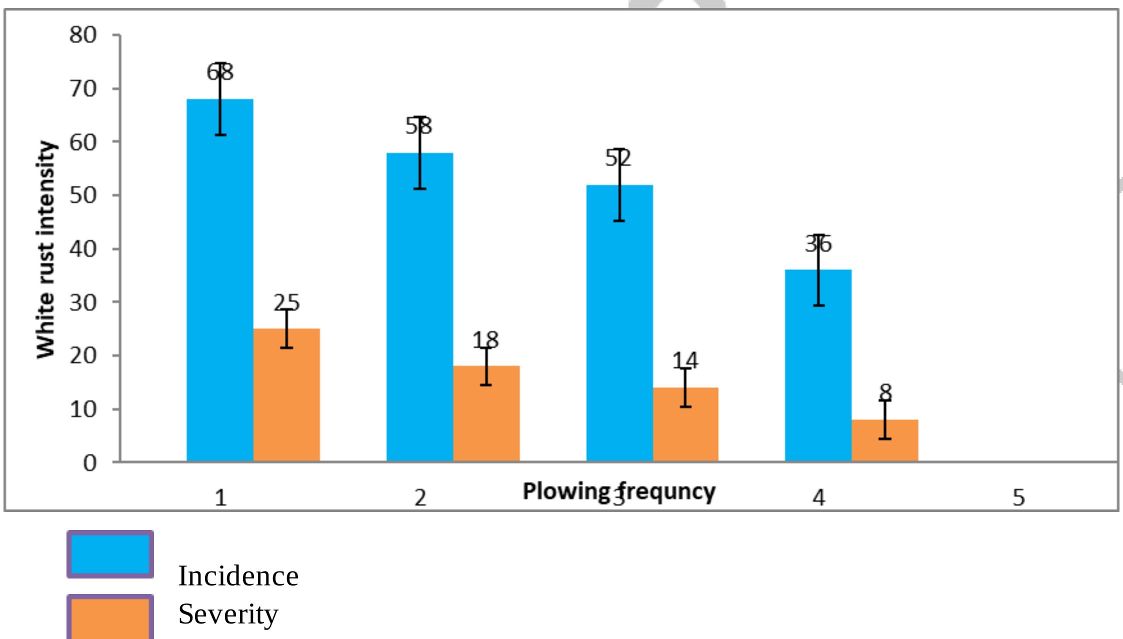


Figure 5: Effects of plowing frequency on white rust intensity

Association of growth stages and altitude with white rust disease

The intensity of white rust was significantly correlated with altitude, but the disease was_not significantly correlated with the growth stages of Ethiopian mustard (Table 9). In the current study, the growth stages of Ethiopian mustard have a great effect on the disease intensity. The disease was more important at the pod growth stage. This indicates at the cotyledon growth stage of the Ethiopian mustard is able to resist the white rust. The results of the present study conform to those reported earlier by Shek *et al.*, (2017) and the concept was related to Agrios, (2005) as the resistance to disease increased as the plant reached maturity. But, we didn't find a correlation between white rust and growth stages. The strong correlation of altitude with white rust indicates that the disease was increased as the elevation of the land increased by m. a. s. l. In this case, the intensity of white rust is high in the highlands of the central part of Oromia. This may be due to the fact that the disease needs the lowest temperature for mustard infection and needs the highest relative humidity (Mehta, 2014). Consequently, the maximum disease incidence (100%) and severity (50.13%) of white was scored at the altitude of 2413 m.a.s.l., which indicates the highland of the survey areas (Table 3). The strong correlation between leaf spot and white rust on mustard resulted in synergistic effects. This result was related to the time response for more than one disease, as both the plant and its efficacy can be reduced during this time (Agrios, 2005).

Table 9: Association of growth stages and altitude with white rust disease

	Disease incidence	Percent severity index	Growth stages	Altitude
Disease incidence	1			
Percent severity index	0.7***	1		
Growth stages	-0.07***	0.3***	1	
Altitude	0.5***	0.6***	0.3ns	1

Pathogenicity test on detached leaves of Ethiopian mustard.

In a laboratory experiment, the Pathogenicity test was carried out by collecting diseased, free-detached leaves of Ethiopian mustard from the greenhouse at Holetta Agricultural Research Center. The injected leaves' infection levels showed a variety of symptoms. The results clearly demonstrated that the isolated original fungus and the artificially re-cultured fungus that was taken from infected leaves shared similarities. Results from a previous study that demonstrated the Pathogenicity of white rust on detached mustard leaves, as detailed by Bisht *et al.* (2018), are comparable with those from this investigation.

Reaction of Ethiopian mustard genotypes against white rust disease

Twenty Ethiopian mustard genotypes were evaluated for host resistance against white rust during the main cropping season of 2021–2022. The reaction of Ethiopian mustard genotypes against isolates of white rust was identified for the first time in Ethiopia. The present study results showed that the percentage of disease incidence and severity in different genotypes of Ethiopian mustard at 1% probability level was significantly different (Table 10). The genotypes showed different levels of resistance to infection. Out of the 20 genotypes (four improved varieties and 16 breeding lines (advanced varieties) with one local cultivar) Ethiopian mustard genotypes used in the experiment, two genotypes (S-67xHoletta-1-9/2/18/2/37/4/1 and Yellow Dodola) were highly resistant, followed by 3 genotypes (Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1, S-67xHoletta-1-9/2/18/2/24/2, and S-67xHoletta-1-9/2/18/2/37/4 were resistant and five genotypes (S-67xHoletta-1-9/2/18/2/45/3, Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3, Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/2, S-67xHoletta-1-9/2/18/2/37/3), were moderately resistant, and the other 10 genotypes were susceptible against white rust (Table 10). However, the genotypes differed in susceptibility as indicated by the incubation period, disease severity, AUDPC, and yield loss reduction. These variations of response among these genotypes might be due to different factors. Since all cultivars were tested under the same environmental conditions, data collected from this study might more truly reflect genetic resistance or susceptibility of the varieties. Among 20 genotypes tested, the least AUDPC value was recorded from S-67xHoletta-1-7/1/13/2/26/2. Overall, the present study concluded that S-67xHoletta-1-9/2/18/2/37/4/1 and Yellow dodola were highly resistant, which can be easily adopted by farmers to control white rust in their cultivated fields.

The mean disease reaction of the comparison of the severity and AUDPC indicated that of "S-67xY.D.2/2/4/1/7/3" was 40% and 2144, respectively, followed by the severity and AUDPC of the second "S-67xHoletta-1-7/1/13/2/26/2" line was 39.7% and 2289, respectively. It was significantly different from all of the other 14 breeding lines and released 4 varieties. Both of those lines were not significantly different from each other. But, the first "S-67xY.D.2/2/4/1/7/3" line was significantly different from the third line, "S-67". The second breeding line, "S-67xHoletta-1-7/1/13/2/26/2", and the third line, "S-67," were not significantly different from each other. The yellow dodola was the best variety from the rest of the lines and varieties. It had the lowest AUDPC (273) and it was highly resistant to white rust, followed by the breeding line, "S-67xHoletta-1-9/2/18/2/37/4/1," which was also highly resistant to white rust and had the lowest AUDPC (311). Both these varieties were not significantly different from each other based on their disease severity (Table 10).

Several reports from several countries urged the use of resistant materials. All *B. napus* accessions and most *B. oleracea* cv are resistant to *A. candida* (Barbetti, 1988; Mukherjee *et al.*, 2001). Three genes, AC-7-1, AC-7-2, and AC-7-3, control resistance in *B. napus* cv. Regent. Tower 1, 2, 3, 4, Gullivar, Midas, Norin, Regent, H-715, and HNS-1 are resistant to *A. candida* in India. Tobin and PYS-6 have been reported to be resistant to *A. candida* in *B. rapa* var. yellow sarson type 6. *B. rapa* var. brown sarson cultivars BSH-1 and BS-15 were shown to be resistant to the white rust disease (Tiwari *et al.*, 1988; Paladhi *et al.,* 1993). In India, all *B. alba* accessions and HC1, 2, 3, 4, and 5 of *B. carinata* have been found to be resistant to *A. candida*. According to Tiwari *et al.* (1988) white rust resistance has been reported in *B. alba*, *B. campestris* (varieties BSH-1,Chamba,Gulivar,Sangam,SSK1,TH-68), *B. carinata* (HC-1), *B. juncea* (varieties DIR-1507,DIR-1522, ZEM-1) and *B. napus* (varieties Tower, GS-7027, HNS-4, HNS). Control of WR through host resistance has been attempted by identifying sources of resistance (Fan *et al.,* 1983; Saharan *et al.,* 1988; Tiwari *et al.,* 1988). All sources of resistance identified so far are race-specific and governed by major genes.

Table 10: Reaction and AUDPC of Ethiopian mustard genotypes against white rust

Genotypes	Mean(disease severity)	Reaction	AUDPC
S-67xY.D.2/2/4/1/7/3	40^{a}	Susceptible	2144
S-67xHoletta-1-7/1/13/2/26/2	39.7^{ab}	Susceptible	2289
S-67	35^{bc}	Susceptible	2000
S-67xHoletta-1-5/2/10/2/20/4	33.7^{cd}	Susceptible	1953
Derash	32.7^{cde}	Susceptible	1931
Local cultivar	32^{cde}	Susceptible	1864
Y.D.xBAR-1030/79-436/2001/6/1/10/1/15/3	32^{cde}	Susceptible	1554
Y.D.xBAR-1030/79-328/2001/8/2/13/1/22/4	29.3^{def}	Susceptible	1706
S-67x34477 Pakistan 5/2/9/2/14/3	29 ^{def}	Susceptible	1703
S-67xY.D.3/1/5/1/9/4	28.3 ^{def}	Susceptible	1650
Tesfa	27.3^{ef}	Susceptible	1581
S-67xHoletta-1-9/2/18/2/45/3	25.3^{fg}	Moderately Resistant	1503
Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/2	22^{g}	Moderately Resistant	1341
S-67xHoletta-1-9/2/18/2/37/3	11.3^{h}	Moderately Resistant	729
Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3	10.3^{hi}	Moderately Resistant	583
Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1	8.7^{hij}	Resistant	475
S-67xHoletta-1-6/2/12/2/24/2	7^{hij}	Resistant	328
S-67xHoletta-1-9/2/18/2/37/4	6.7^{hij}	Resistant	389
S-67xHoletta-1-9/2/18/2/37/4/1	5.3^{ij}	Highly Resistant	311
Yellow dodola	4.7^{j}	Highly Resistant	273

Yield differences of Ethiopian mustard genotypes against white rust

According to the ANOVA model, the Ethiopian mustard yield was significantly different (Table 11). The mean comparison also showed that the Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3 line had the highest yield (3939kg/ha), which was not significantly different from the S-67x34477 Pakistan 5/2/9/2/14/3 and S-67xHoletta-1-9/2/18/2/45/3 lines, but significantly different from the S-67, which had yields of 2757 kg/ha. Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1 yielded 851kg/ha, while S-67xHoletta-1-9/2/18/2/37/4/1 yielded 923kg/ha (Table 11).

Table 11: Mean comparison of yields of Ethiopian mustard genotypes

S. No	Genotypes	Yield
1	Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/3	3939^{a}
2	S-67x34477 Pakistan 5/2/9/2/14/3	3164^{ab}
3	S-67xHoletta-1-9/2/18/2/45/3	3058^{abc}
4	S-67	2757^{bcd}
5	Yellow dodola	2657^{bcde}
6	Y.D.xBAR-1030/79-328/2001/8/2/13/1/22/4	2587^{bcde}
7	Y.D.xBAR-1030/79-436/2001/6/2/11/1/18/2	2514^{bcde}
8	S-67xY.D.3/1/5/1/9/4	2238^{bcdef}

9	S-67xHoletta-1-9/2/18/2/37/3	2020^{bcdefg}
10	Local	1998^{bcdefg}
11	Derash	1895^{cdefg}
12	S-67xY.D.2/2/4/1/7/3	1895^{cdefg}
13	Tesfa	1837^{cdefg}
14	S-67xHoletta-1-6/2/12/2/24/2	1754^{defg}
15	S-67xHoletta-1-9/2/18/2/37/4	1671^{defg}
16	S-67xHoletta-1-5/2/10/2/20/4	1434^{efg}
17	Y.D.xBAR-1030/79-436/2001/6/1/10/1/15/3	1195^{fg}
18	S-67xHoletta-1-7/1/13/2/26/2	1192^{fg}
19	S-67xHoletta-1-9/2/18/2/37/4/1	923^{g}
20	Y.D.xBAR-1029/79-436/2002/9/2/15/1/28/1	851^{h}

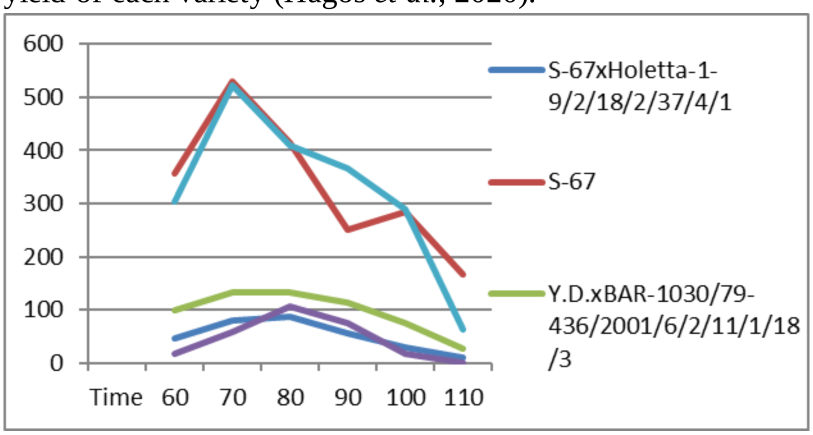
Table 12. Association of white rust with yield on Ethiopian mustard genotypes

As Karl Pearson correlation showed that the white rust severity and disease Progress Curve were not significantly correlated with yield (Table 12).

Table 12: The correlation of three variables during genotypes evaluation

	Yield	Percent severity index	Disease under progress curve
Yield	1		
Percent severity index	0.09	1	
Disease under progress curve	0.06ns	-0.5***	1

The disease severity and AUDPC were classified as the response of Ethiopian mustard against white rust disease. Then, the ANOVA model showed us the significant difference between Ethiopian mustard and their response to white rust. Ten genotypes of Ethiopian mustard were susceptible to white rust, whereas two and three genotypes were classified as highly resistant and resistant to this disease (Figure 6). The results of the present study conform to those reported earlier by Bisht *et al.* (1994), Gupta and Saharan (2002), and Bisht *et al.* (2018). The Karl Pearson correlation showed that the white rust severity and disease progress curve were not significantly correlated with yield. This indicates that the increment of disease severity may not affect the yield of each variety (Hagos *et al.*, 2020).



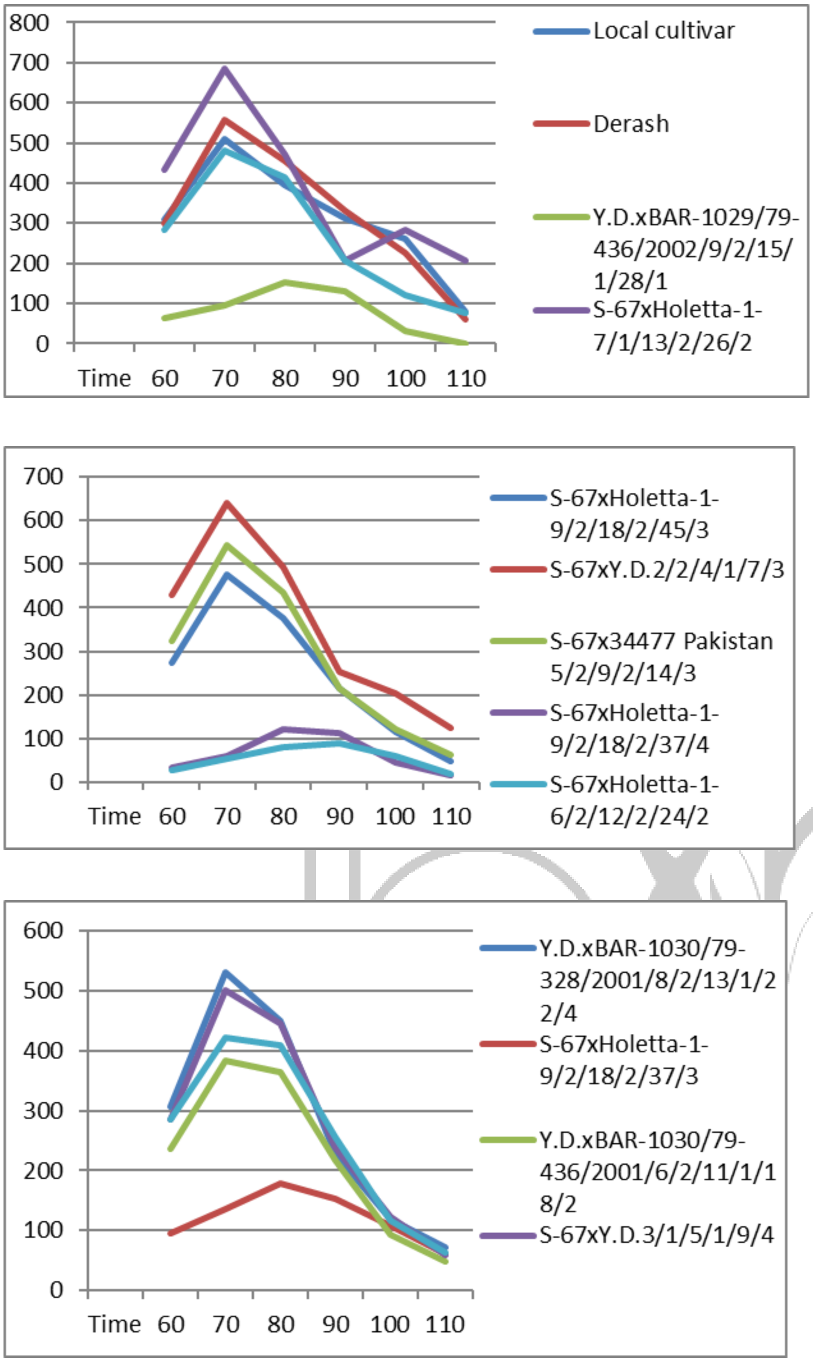


Figure 6. White rust progress curves on Ethiopian mustard genotypes against white rust.

CONCLUSIONS

White rust was assessed in all the study fields in Dendi district, with a prevalence of 100%, and in Welmera, Jeldu, and Dire Incini districts, with a prevalence of 93.3, 86.6, and 80%, respectively. The incidence of white rust varied from 40.8-100% and the severity index ranged from 4–50.13%. The maximum level of white rust incidence and severity was recorded at a high altitude level of 2413 m.a.s.l. in the study areas. All the evaluated 20 Ethiopian mustard genotypes exhibited highly resistant, resistant, moderately resistant, and susceptible reactions to the disease. There is no complete resistance (zero infection types) observed among the evaluated Ethiopian mustard genotypes. But their susceptibility level varies from variety to variety. Overall, the present study concluded that S-67xHoletta-1-9/2/18/2/37/4/1 and Yellow dodola were highly resistant, which can be easily adopted by the farmers to control white rust in their cultivated fields. White rust disease epidemics is highly influenced by climatic change and incited by a complex of *Albugo* species, efforts should be made towards the integration of multiple control strategies to the disease. The current study suggested that the genetic diversity of the pathogen needs further attention to study the management practices. This study could furthermore recommend the biological management and IPM approaches for the control of Ethiopian mustard white rust, which should be environmentally sound and effective ways of eliminating white rust.

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