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# Interaction of varieties and fungicides across seasons and locations for the control of Asian soybean rust (*Phakopsora pachyrhizi*) in Southwestern Ethiopia

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### ABSTRACT

Soybean is emerging as an important legume and oil crop in Ethiopia. However, the crop has been facing serious threats from Asian Soybean Rust (ASR), causing significant damage to the crop and not allowing it to realize its potential. This study aimed to determine the interaction of three varieties with various fungicide treatments across two seasons and two locations to manage ASR. The experiment was laid out in a RCBD in a factorial [three soybean varieties (V) and six fungicides (F)] treatment combination at two locations (L) for two years (Y) with three replications. The analysis of variance across seasons revealed highly significant V  $\times$  F  $\times$  Y interactions for grain yield, and F  $\times$  Y interactions for severity (%) and AUDPC; while efficacy was significant (P = 0.05). The V  $\times$  Y interaction showed highly significant differences for AUDPC. Across locations, there were highly significant (P = 0.05) for the V  $\times$  F interaction. There were two rates (0.75 and 1 l ha^{-1}) of the fungicide Opera Max that provided the best protection against ASR across locations, varieties, and seasons. The yield loss on the unsprayed control treatment ranged between 29 and 65%. The evidence generated from this study clearly demonstrates the importance of identifying the right variety, rates and types of fungicides for the control of ASR for higher production and productivity of soybean in Ethiopia.

### 1. Introduction

Soybean is an important and highly demanded crop globally (Santos, 2019). It is the most traded legume, accounting for about 85% of the global grain legume trade (Abate et al., 2011). In Africa, soybean production has been progressively increasing since the early 90's (Chigeza et al., 2019; FAO, 2021). Cornelius and Goldsmith (2019) attributed most of such increase to the expansion in the areas of production, not productivity; however, Chigeza et al. (2019) reported comparable contributions of both areas of production and yields. Regardless of the long history and increasing production trend, several authors asserted that soybean productivity in Africa is still very low compared to other regions (Cornelius and Goldsmith, 2019; Diers and Scaboo, 2019; Santos, 2019) (Chigeza et al., 2019).

The average productivity of soybean in Ethiopia is 2.3 t ha<sup>-1</sup> (CSA,

2021; FAO, 2021), which is low relative to the global average productivity of 2.7 t ha<sup>-1</sup> and productivity in the US and Brazil (3.4 t ha<sup>-1</sup>) in 2018 (FAO, 2021). Several production constraints contribute to this low productivity. Diseases, especially Asian soybean rust (ASR) caused by the obligate fungal pathogen *Phakopsora pachyrhizi*, are the most important production limiting factors (Tesfaye et al., 2016). ASR is a highly destructive foliar disease affecting soybean productivity in several countries worldwide (Mueller et al., 2009). Juliatti et al. (2017) reported its first occurrence in Africa in 1997. However, Caldwell and Laing (2002) reported that the first introduction to Africa occurred around 1975. To date, ASR is reported in 11 African countries, including relatively recent reports in Ethiopia (Murithi et al., 2016; Tesfaye et al., 2016). The disease was first reported in Ethiopia in 2017, though it was first spotted in 2011 (Tesfaye et al., 2016). Since its occurrence in Ethiopia, ASR has been causing significant yield reduction, and

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currently, more than 83,000 ha cropped to soybean are estimated to be at risk (CSA, 2021).

Tropical climates (high temperature and humidity) are ideal for ASR development, especially during the dry season (Krisnawati et al., 1970). Climate change is expected to intensify the yield losses from ASR by changing the host plant physiology and resistance, which might also influence the pathogen epidemiology (Sivakumar, 2008). The yield losses from ASR vary from country to country, and the tolerance level of the varieties used in any given country dictates the yield losses. Sinclair and Hartman (1999) and Hartman et al. (1991) reported 40%-90% yield loss in some Asian countries. Yorinori et al. (2005) reported a 30%-75% yield loss in Brazil and Paraguay. Shin and Tschanz (1986) reported 22% and 69% yield losses in tolerant and susceptible varieties, respectively. Hartman et al. (1991), Bromfield (1984), and Yang et al. (1991) reported that yield losses might reach up to 80% under severe ASR outbreaks because of premature leaf senescence that affects proper pod filling. Furthermore, losses might reach up to 100% in the absence of any chemical control measures, coupled with weather conditions, variety's susceptibility and their physiology (Tukamuhabwa and Maphosa,

Rupe (2008) recommended three approaches for controlling ASR: fungicides application, genetic resistance, and cultural practices. The use of resistant genotypes is an effective strategy to reduce soybean yield losses due to ASR (Kendrick et al., 2011). However, this approach is considered a long term strategy (Rupe, 2008) because of both lack of varieties with effective resistance to the disease (Vuong et al., 2016) and in most cases, disease resistances is controlled by a single gene that often lacks durability (Vuong et al., 2016). In addition, the potential for the presence or development of different races with variable pathogenicity, virulence and genetic composition complicates the effectiveness of resistant varieties for the control of ASR (Murithi et al., 2016). The use of chemical fungicides has been reported to manage ASR effectively. However, the effectiveness of fungicides depends on early detection of the disease and identification of the correct application time and rate (Delaney et al., 2018; Rupe, 2008). Fungicides applied for the control of ASR can also protect the crop from other fungal diseases, such as aerial web blight (Cercospora kikuchii), frog eye leaf spot (Cercopsora sojina), pod and stem blight (Diaporthe phaseolorum var. sojae), and target spot (Corynespora cassiicola) (Allen et al., 2014).

Quinone outside inhibitors (QoI; strobilurins) and demethylation inhibitors (triazoles) are two classes of fungicides commonly used in the US to control ASR. Mixtures of different classes of fungicides have also been used (Delaney et al., 2018; Juliatti et al., 2017). Strobilurins (e.g., azoxystrobin, pyraclostrobin, and trifloxystrobin) have a protectant property preventing penetration and establishment of the pathogen on the host tissue, but with little or no effect after establishment or colonization of the pathogen (Tenuta et al., 2008). Triazole fungicides such as flutriafol, tetraconazole, triadimenol, epoxiconazole, tebuconazole, propiconazole, hexaconazole, and difenoconazole have both protectant and curative properties that prevent the progression of the infection and also interferes with sporulation, therefore inhibiting the reproduction of the pathogen (Tenuta et al., 2008).

In Ethiopia, several synthetic fungicides have been evaluated and registered for use on various crops, especially for the management of wheat rust (Bekana, 2020; Mengesha, 2020). However, despite the increasing trend of soybean production and high yield losses due to ASR, evaluation of fungicides to establish recommendations for the control of ASR have not been conducted. Hence, this study was designed to evaluate three rates of Opera Max (85 g l $^{-1}$  F500 and 62.5 g l $^{-1}$  epoxiconazole) fungicide. Opera Max which was found effective in managing ASR in Brazil (Juliatti et al., 2017), is registered in Ethiopia for the control of rusts, and is also recommended for the control of ASR. The objectives of this study were to determine the interaction of fungicide treatments with varieties across locations and seasons with a view to identify the fungicide and soybean variety combination providing the greatest control of ASR. Results from the current study will provide

valuable information to increase the productivity of soybean by reducing the effects of ASR.

#### 2. Materials and methods

### 2.1. Fungicides tested

Apart from Opera Max (85 g l $^{-1}$  F500 and 62.5 g l $^{-1}$  epoxiconazole), produced by BASF, two other fungicides i.e., Nativo SC 300 (trifloxystrobin + tebuconazol) registered in Ethiopia for the control of rust and fungal diseases in wheat, onion and tomato, and Luna Sensation SC 500 (flopyram + trifloxystrobin) registered in Ethiopia for the control of powdery mildew in pepper, were included in the study as reference chemicals for comparison. Both Nativo SC and Luna Sensation are products of Bayer.

### 2.2. Experimental materials and treatments

The experimental materials include three officially released soybean varieties (Clark 63 K, Afgat, and SCS-1). The fungicide treatments include three rates of Opera Max (0.5, 0.75, and 1 l ha $^{-1}$ ), Luna Sensation SC 500 at manufacturer recommendation of 0.15 l ha $^{-1}$ , and Nativo SC 300 at the manufacturer recommendation of 0.5 l ha $^{-1}$ . These treatments were compared with the control (unsprayed) soybean plants.

The experiment was conducted during two seasons (2017 and 2018) and sown in June each year. Application of fungicide was conducted at flowering stage when more than 5% incidence of ASR was observed in the field. A manual knapsack sprayer with a capacity of  $16\,L$  (KS-16) was used for fungicides application by thoroughly wetting the underside of the leaves. Fungicide application was done three times with spray intervals of 7–9 days.

### 2.3. Experimental sites description

The trial was conducted at Jimma Agricultural Research Center (JARC) and Mettu Agricultural Research Sub-Center (MARSC) in southwestern Ethiopia. These two locations are ASR hotspots, where the disease has been naturally occurring since 2011 (Tesfaye et al., 2016). It is located at a latitude of 7°46′N, longitude of 36°47′E and an altitude of 1,750 masl. The center has the maximum (26.3 °C) and minimum (11.6 °C) temperature and receives 1,530 mm average rainfall per annum. The center is situated in sub-humid tepid to cool mid-highland agroecology. MARSC is located in the Illuababora zone of Oromia Regional State at a latitude of 8°19′N, longitude of 36°35′E and an altitude of 1,580 masl. The center is situated in the country's sub-humid tepid to cool mid-highland agroecology.

### 2.4. Experimental design and plot

The treatments were arranged in a factorial combination of three varieties with six fungicide sprays in a randomized complete block design (RCBD), forming 18 treatment combinations, including the control. The plot size was 4 m  $\times$  2.4 m with four rows (with two harvestable central rows) and 60 cm  $\times$  5 cm inter and intra-rows spacings, respectively. The recommended cultural practices were uniformly applied to all the treatments.

### 2.5. Data collection

Data on disease incidence and severity were collected from 10 randomly selected and tagged plants from the two central rows of each plot at both Jimma and Mettu in 2018, and only at Mettu in 2017. The disease data was not collected at Jimma in 2017 because ASR did not occur during that year. The ASR disease data was collected six times at every two weeks (14 days) intervals. The data collection started before spraying and continued after spraying. Severity scores and percent

ranges were used to determine % disease severity. A predetermined scale was used for visual estimation based on a 0–5 scale during data collection. Accordingly, 0 = No disease, 1 = Trace, 1-5%; 2 = 10-20% tolerant; 3 = 25-40% moderately susceptible; 4 = susceptible, 40-60% and 5 = highly susceptible, >80. Data on disease severity was recorded as the percentage of leaf area covered by the lesion (Vincelli and Hershman, 2011).

The plants were harvested at maturity and yield data was collected from 10 randomly selected plants from the two middle rows. Seed moisture content was recorded and used to adjust grain yield for 12.5% standard moisture content of soybean. In addition, 100-seed weight, was collected.

### 2.6. Data analysis

The area under the disease progress curve (AUDPC) was calculated from the disease severity percentages using the standard interactive procedures (Shaner and Finney 1977).

The AUDPC was calculated as AUDPC = 
$$\sum_{i=1}^{n-1} \frac{\pi(\mathrm{Yi} + \mathrm{Y}_{i+1})(\mathrm{X}_{i+1} - \mathrm{X}_i)}{2}$$

where: X = is the time in days; Y = is the proportion of diseased leaves (cumulative severity = xct), I = is the ith observation, i+1 = is the next observation, n = is the total number of observations,  $X_i = is$  the time on the first observation ( $x_i = 0$  time).

Transformation of disease scoring and count data was performed to ensure the normality of data (Gomez and Gomez, 1984). Test of homogeneity of error variances for each of the individual locations (Jimma and Mettu) and seasons (2017 and 2018) was performed for all the traits using F-test as suggested for test of homogeneity of two error variances (Gomez and Gomez 1984). ANOVA was performed for across locations data collected from Jimma and Mettu in 2018, while across years, 2017 and 2018, performed for the data collected from Mettu alone. The data in 2017 at Jimma was not recorded because ASR did not occur. The ANOVA was performed using SAS software version 9.4 (SAS, 2013). The mean separations were performed using Duncan's Multiple Range Test (DMRT,  $\alpha=0.05$ ).

Fungicide efficacy (%) was calculated using Abbott's formula (Abbott 1925),

Efficacy (%) = 
$$\frac{X - Y}{X} \times 100$$

where X – disease severity in the control treatment; Y – disease severity in fungicide treatment.

While yield loss (YL) was estimated using the following formula:

$$YL(\%) = \frac{X - Y}{X} \times 100$$

where X = Maximum yield obtained from the treatment; Y = Unsprayed control treatment.

#### 3. Results

The result of test of homogeneity revealed that the error variances for all the traits across the two seasons were homogenous; while all the traits [100 seed weight, severity (%), efficacy (%) and AUDPC], except grain yield showed non-homogenous error variances across the two locations. Hence, the combined analysis of variance was performed for all the traits across the two seasons (2017 and 2018), and only for grain yield across locations.

Accordingly, the ANOVA for each location revealed a significant variety  $\times$  fungicide interaction only for AUDPC at Mettu (Table 1). The main effects of varieties and fungicides showed highly significant (P < 0.01) differences for 100-seed weight, severity, efficacy and AUDPC, except the varieties that showed significant (P < 0.05) differences at Jimma. At Mettu, each of the varieties and fungicides showed highly significant differences for 100-seed weight, severity and efficacy, except for the varieties that showed non-significant differences in efficacy.

The across seasons combined ANOVA revealed highly significant three-way interactions of V  $\times$  F  $\times$  Y for grain yield at Mettu (Table 2). The F  $\times$  Y interaction was also highly significant for severity (%) and AUDPC, while efficacy (%) showed significant differences (P < 0.05). The V  $\times$  Y interaction was highly significant (P < 0.01) for severity and AUDPC. The fungicide treatments showed highly significant differences for all the studied traits, i.e., grain yield, 100-seed weight, severity (%),

**Table 2**ANOVA for the main and interaction effects of varieties and fungicides for grain yield, 100-seeds weight (HSW), disease severity, fungicide efficacy, and AUDPC during two years (2017 and 2018) for the management of Asian soybean rust at Mettu, Ethiopia.

| , .   |     |   |                         |                          |                 |                    |
|---|-----|---|-------------------------|--------------------------|-----------------|--------------------|
| Sources of variation  | Dfª | Yield (t<br>ha <sup>-1</sup> ) <sup>b</sup> | HSW<br>(g) <sup>c</sup> | Severity<br>(%)          | Efficacy<br>(%) | AUDPC <sup>c</sup> |
| Varieties<br>(Var)  | 2   | 0.09*                                       | 53.2<br>**              | 265.6<br>(136.0)<br>**   | 222.1 ns        | 677,179 **         |
| Year (Y)  | 1   | 0.38**                                      | 527.0<br>**             | 781.9<br>(379.8)<br>**   | 28.5 ns         | 1,230,165**        |
| $Var \times Y \\$   | 2   | 0.20**                                      | 2.6 ns                  | 1,46.7<br>(72.7)**       | 294.7 ns        | 409,572 **         |
| Fungicides<br>(Fung)  | 5   | 3.74**                                      | 42.4<br>**              | 1,585.0<br>(825.7)<br>** | 12,898.3<br>**  | 4,147,649<br>**    |
| $Var \times Fung$   | 10  | 0.12**                                      | 2.3 ns                  | 38.2<br>(12.8)ns         | 37.1 ns         | 108,555 *          |
| $Fung \times Y$   | 5   | 0.19**                                      | 3.4 ns                  | 123.1<br>(56.28)<br>**   | 364.6 *         | 354,522 **         |
| $\begin{array}{c} \text{Var} \times \text{Fung} \\ \times \text{Y} \end{array}$ | 10  | 0.09**                                      | 3.0 ns                  | 25.9<br>(10.5)ns         | 65.9 ns         | 64,853 ns          |
| Error   | 68  | 0.025                                       | 1.7                     | 15.6<br>(8.5)            | 117.2           | 43,711             |

<sup>&</sup>lt;sup>a</sup> Df: degrees of freedom.

Table 1

ANOVA of individual locations (Mettu and Jimma) for the main and interaction effects of varieties (Var) and fungicides (Fung) for the management of Asian soybean rust for some of the traits with non-homogenous variances.

| Source of variations df |    | Mettu            |                   |              |                    | Jimma   |                |              |            |
|-------------------------|----|------------------|-------------------|--------------|--------------------|---------|----------------|--------------|------------|
|                         |    | HSW <sup>a</sup> | Severity (%)      | Efficacy (%) | AUDPC <sup>b</sup> | HSW (g) | Severity (%)   | Efficacy (%) | AUDPC      |
| Var                     | 2  | 38.3**           | 403.6 (203.8)**   | 107.3 ns     | 1,065,898 **       | 16.3 ** | 8.3 (5.4)*     | 255.17**     | 2,1650 **  |
| Fung                    | 5  | 15.2**           | 1,223.7 (607.0)** | 7,446.5 **   | 3,305,225 **       | 26.7 ** | 102.5 (66.9)** | 2937.96**    | 158,481 ** |
| Var*Fung                | 10 | 4.9ns            | 55.9 (19.5)ns     | 71.0 ns      | 145,667 *          | 0.8 ns  | 1.6 (0.8)ns    | 42.58ns      | 1,466 ns   |
| Error                   | 34 | 2.5              | 23.3 (12.3)       | 153.8        | 50,312             | 0.6     | 1.6 (1.0)      | 30.44        | 2,248      |

<sup>&</sup>lt;sup>a</sup> HSW = Weight in grams of 100 seeds. A single asterisk (\*) indicates P < 0.05, while two asterisks (\*\*) indicate P < 0.001. ns: not significant.

<sup>&</sup>lt;sup>b</sup> A single asterisk (\*) indicates P < 0.05, while two asterisks (\*\*) indicate P < 0.01, ns: non significant.

<sup>&</sup>lt;sup>c</sup> AUDPC: area under disease progress curve.

<sup>&</sup>lt;sup>b</sup> AUDPC = Area under disease progress curve.

efficacy (%), and AUDPC. The across locations combined ANOVA revealed highly significant (P < 0.01) three-way interactions of F  $\times$  V  $\times$  L for grain yield (Table 3).

## 3.1. Response of grain yield to variety and fungicides across seasons and locations for the management of ASR

The highest yield of 3.4 t ha $^{-1}$  was obtained in response to Opera Max (1 l ha $^{-1}$ ) treatment on Clark 63 K in 2018 (Table 4). This fungicide–variety-year combination was statistically similar with the application of Opera Max (1 l ha $^{-1}$ ) on Afgat and SCS-1 (3.3 t ha $^{-1}$ ); Opera Max (0.75 l ha $^{-1}$ ) on Clark 63 K, Afgat and SCS-1 with respective yields of 3.1 t ha $^{-1}$ , 3.1 t ha $^{-1}$  and 3.2 t ha $^{-1}$ ; Opera Max (0.5 l ha $^{-1}$ ) on Clark 63 K (3.0 t ha $^{-1}$ ), Afgat (3.0 t ha $^{-1}$ ), Nativo on Afgat (3.1 t ha $^{-1}$ ) and SCS-1 (3.2 t ha $^{-1}$ ), and Luna Sensation on Afgat (3.0 t ha $^{-1}$ ) in 2018. Similarly, this highest yielding fungicide–variety-year treatment combination was also not significantly different from the application of Opera Max (1 l ha $^{-1}$ ) on Clark 63 K (3.2 t ha $^{-1}$ ), Afgat (3.1 t ha $^{-1}$ ), and SCS-1 (3.0 t ha $^{-1}$ ); Opera Max (0.75 l ha $^{-1}$ ) on Clark 63 K (3.1 t ha $^{-1}$ ), and Afgat (2.9 t ha $^{-1}$ ), and Opera Max (0.5 l ha $^{-1}$ ) on Clark 63 K (2.9 t ha $^{-1}$ ) in 2017.

Although the application of Opera Max ( $1\,l\,ha^{-1}$ ) gave the maximum yields of 3.54 t  $ha^{-1}$  at Jimma on the variety Afgat, and 3.39 t  $ha^{-1}$  at Mettu on the variety Clark 63 K, most of the fungicide treatments were not significantly different for yield across locations and varieties, except some of the fungicides and the control treatments (Table 5). At Jimma, all the fungicide treatments showed statistically similar yield, except Luna Sensation and the control treatments on all the varieties and Opera Max (0.5  $l\,ha^{-1}$ ) and Nativo on SCS-1 variety. Similarly, all the fungicide treatments at Mettu, except the control treatment on all the varieties, and Nativo and Luna Sensation on Clark 63 K produced statistically similar yields with the highest yielding genotype.

## 3.2. Response of 100-seeds weight to varieties and fungicides for the management of ASR across seasons and locations

In 2017, the 100 seeds weight was generally higher at Jimma than at Mettu (Fig. 2). At Jimma, the highest 100-seeds weight of 17.0 g was recorded on the variety Clark 63 K, while the 100 seed weights of Afgat (15.1 g), and SCS-1 (15.1 g) were the same. At Mettu, Afgat gave the lowest 100 seed weight of 9.2 g, while SCS-1 (11.5 g) and Clark 63 k (11.9 g) produced nearly the same 100-seeds weights.

In 2018, the highest 100-seeds weight of 17.7 and 17.4 g were recorded for the application of Opera Max (1 l ha $^{-1}$ ) and Nativo, followed by Opera Max at the rates of 0.75 and 0.5 l ha $^{-1}$ , with respective 100-seed weights of 16.3 and 16.2 g at Jimma (Table 6). The lowest 100-seeds weight was recorded for the unsprayed control treatment (13.0 g), followed by Luna Sensation (15.0 g). At Mettu, all the fungicides applications, except the control treatment, showed statistically non-

**Table 3**ANOVA for the main and interaction effects of varieties and fungicides for grain yield in response to the management of Asian soybean rust disease across two locations (Jimma and Mettu).

| Sources of variation         | df <sup>a</sup> | Grain yield (t ha <sup>-1</sup> ) <sup>b</sup> |
|------------------------------|-----------------|--|
| Varieties (Var)              | 2               | 0.83**   |
| Location (Loc)               | 1               | 0.40**   |
| $Var \times Loc$             | 2               | 0.74**   |
| Fungicide (Fung)             | 5               | 34.59**  |
| Var × Fung                   | 10              | 0.97ns   |
| Fung × Loc                   | 5               | 1.13**   |
| $Var \times Fung \times Loc$ | 10              | 1.94**   |
| Error                        | 48              | 0.052  |

a df: degrees of freedom.

**Table 4**Mean value of grain yield of soybean in response to the effects of varieties and fungicides for the management of ASR across two years (2017 and 2018) at Mettu.

| Year  | Fungicide              | Yield (t ha <sup>-1</sup> ) <sup>a</sup> |                        |                    |  |  |
|-------|------------------------|--|------------------------|--------------------|--|--|
|       |                        | Clark 63 K                               | Afgat                  | SCS-1              |  |  |
| 2017  | Control                | $2.0 \pm 0.11$ ijk                       | $2.2\pm0.09$ hij       | $2.0 \pm 0.03$ ijk |  |  |
|       | Luna Sensation SC      | $2.4~\pm$                                | $2.4~\pm$              | $2.4~\pm$          |  |  |
|       | 500 (0.15 lt/ha)       | 0.07fghi                                 | 0.02fghi               | 0.09fghij          |  |  |
|       | Nativo SC 300 (0.5 lt/ | $2.7~\pm$                                | 2.7 $\pm$              | 2.7 $\pm$          |  |  |
|       | ha)                    | 0.1cdefg                                 | 0.13defgh              | 0.11defgh          |  |  |
|       | Opera max (0.5 lt/ha)  | $2.9 \pm$                                | $2.7~\pm$              | $2.6 \pm$          |  |  |
|       |                        | 0.08abcdef                               | 0.06cdefgh             | 0.02efgh           |  |  |
|       | Opera max (0.75 lt/    | $3.1~\pm$                                | $2.9~\pm$              | $2.8~\pm$          |  |  |
|       | ha)                    | 0.12abcde                                | 0.09abcdef             | 0.04bcdef          |  |  |
|       | Opera max (1 lt/ha)    | $3.2 \pm 0.15 abc$                       | $3.1~\pm$              | $3.0~\pm$          |  |  |
|       |                        |  | 0.15abcd               | 0.07abcde          |  |  |
| 2018  | Control                | $1.9 \pm 0.13$ jk                        | $1.9 \pm 0.04$ ijk     | $1.5 \pm 0.11$ k   |  |  |
|       | Luna Sensation SC      | 2.3 $\pm$                                | $3.0 \pm$              | 2.9 $\pm$          |  |  |
|       | 500 (0.15 lt/ha)       | 0.08ghij                                 | 0.04abcde              | 0.08bcdef          |  |  |
|       | Nativo SC 300 (0.5 lt/ | 2.4 $\pm$                                | $3.1~\pm$              | $3.2~\pm$          |  |  |
|       | ha)                    | 0.06fghi                                 | 0.04abcde              | 0.1abcd            |  |  |
|       | Opera max (0.5 lt/ha)  | $3.0 \pm$                                | $3.0 \pm$              | $2.8 \pm$          |  |  |
|       |                        | 0.11abcde                                | 0.05abcde              | 0.05bcdef          |  |  |
|       | Opera max (0.75 lt/    | 3.1 $\pm$                                | $3.1~\pm$              | 3.2 $\pm$          |  |  |
|       | ha)                    | 0.07abcde                                | 0.04abcde              | 0.16abcd           |  |  |
|       | Opera max (1 lt/ha)    | $3.4\pm0.07a$                            | $3.3\pm0.12~\text{ab}$ | $3.3\pm0.14$       |  |  |
|       |                        |  |                        | ab                 |  |  |
| Grand | mean                   | 2.73                                     |                        |                    |  |  |
| CV%z  |                        | 5.83                                     |                        |                    |  |  |

<sup>&</sup>lt;sup>z</sup> CV = coefficient of variation.

**Table 5**Mean grain yield and AUDPC values of soybean in response to different varieties and fungicide treatments for the control ASR across two locations (Jimma and Mettu) in the 2017 crop season.

| Locations           | Fungicides   | Grain yield t ha <sup>-1a</sup> |                  |                     |  |
|---------------------|--------------|---------------------------------|------------------|---------------------|--|
|                     |              | Clark 63 K                      | Afgat            | SCS-1               |  |
| Mettu               | Control      | 1.90 ±                          | 1.92 ±           | 1.49 ± 0.11klm      |  |
|                     |              | 0.13ijklm                       | 0.04ijklm        |                     |  |
|                     | Luna         | $2.29~\pm$                      | $3.01~\pm$       | $2.86~\pm$          |  |
|                     | Sensation    | 0.08fghij                       | 0.04abcdef       | 0.08abcdefg         |  |
|                     | Nativo       | 2.43 $\pm$                      | 3.07 $\pm$       | $3.15~\pm$          |  |
|                     |              | 0.06efghij                      | 0.04abcde        | 0.10abcde           |  |
|                     | Opera Max    | $2.97~\pm$                      | 2.96 $\pm$       | 2.84 $\pm$          |  |
|                     | (0.5 lt/ha)  | 0.11abcdef                      | 0.05abcdef       | 0.05abcdefgh        |  |
|                     | Opera Max    | 3.07 $\pm$                      | 3.06 $\pm$       | 3.18 $\pm$          |  |
|                     | (0.75 lt/ha) | 0.07abcde                       | 0.04abcde        | 0.16abcde           |  |
|                     | Opera Max (1 | $3.39 \pm 0.07$                 | $3.29~\pm$       | $3.30 \pm 0.14$ abo |  |
|                     | lt/ha)       | ab                              | 0.12abcd         |                     |  |
| Jimma               | Control      | $\overline{1.23\pm0.09m}$       | 1.75 ±           | $1.34\pm0.07lm$     |  |
|                     |              |                                 | 0.15jklm         |                     |  |
|                     | Luna         | 2.18 $\pm$                      | $2.54 \pm$       | 2.10 $\pm$          |  |
|                     | Sensation    | 0.26ghijk                       | 0.19efghi        | 0.06hijkl           |  |
|                     | Nativo       | $3.41\pm0.12$                   | $2.87~\pm$       | 2.70 $\pm$          |  |
|                     |              | ab                              | 0.27abcdefg      | 0.16bcdefgh         |  |
|                     | Opera Max    | 2.89 $\pm$                      | 2.93 $\pm$       | $2.57~\pm$          |  |
|                     | (0.5 lt/ha)  | 0.16abcdefg                     | 0.13abcdefg      | 0.13cdefghi         |  |
|                     | Opera Max    | $3.16 \pm$                      | $3.20 \pm$       | $2.89\ \pm$         |  |
|                     | (0.75 lt/ha) | 0.11abcde                       | 0.02abcd         | 0.03abcdefg         |  |
|                     | Opera Max (1 | $3.50\pm0.22a$                  | $3.54 \pm 0.08a$ | 3.17 $\pm$          |  |
|                     | lt/ha)       |                                 |                  | 0.04abcde           |  |
| Grand mean          | n            | 2.71                            |                  |                     |  |
| CV (%) <sup>z</sup> |              | 8.37                            |                  |                     |  |

 $<sup>^{</sup>z}$  CV = coefficient of variation.

 $<sup>^{\</sup>rm b}$  A single asterisk (\*) indicates P<0.05, while two asterisks (\*\*) indicate P<0.001. ns: not significant.

<sup>&</sup>lt;sup>a</sup> Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, and values sharing the same letter are not statistically different.

<sup>&</sup>lt;sup>a</sup> Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.

**Table 6**Mean value of 100-seed weight (HSW), severity (%), efficacy (%), area under disease progress curve (AUDPC) of soybean in response to the application of fungicides for the management of ASR at Jimma.

| Fungicide                | HSW <sup>a</sup>          | Severity (%)  | AUDPC        |
|--------------------------|---------------------------|---------------|--------------|
| Control                  | $12.98 \pm 0.28 \text{d}$ | 18.64 (25.38) | 733.89 $\pm$ |
|                          |                           | $\pm 0.92a$   | 36.55a       |
| Luna Sensation (0.15 lt/ | $15.01\pm0.38c$           | 16.53 (23.96) | 660.56 $\pm$ |
| ha)                      |                           | $\pm 0.65b$   | 26.25b       |
| Nativo (0.5 lt/ha)       | $17.38\pm0.28$            | 13.22 (21.24) | 543.89 $\pm$ |
|                          | ab                        | $\pm 0.87c$   | 48.97c       |
| Opera max (0.5 lt/ha)    | $16.23\pm0.44\text{b}$    | 11.24 (19.58) | 443.33 $\pm$ |
|                          |                           | $\pm 0.33$ d  | 12.22d       |
| Opera max (0.75 lt/ha)   | $16.26\pm0.5b$            | 10.89 (19.23) | 432.78 $\pm$ |
|                          |                           | $\pm 0.49d$   | 18.71d       |
| Opera max (1 lt/ha)      | $17.67 \pm 0.34a$         | 10.44 (18.8)  | 417.22 $\pm$ |
|                          |                           | $\pm 0.56d$   | 21.23d       |
| CV (%) <sup>z</sup>      | 5.03                      | 9.39 (4.83)   | 8.8          |
| Mean                     | 15.92                     | 13.5 (21.36)  | 538.61       |

<sup>&</sup>lt;sup>z</sup>CV (%) = coefficient of variation.

significantly different 100-seeds weight (Table 7).

### 3.3. Yield loss (%) in soybean following the management of ASR using varieties and fungicides across seasons and locations

The highest yield loss of 55% was recorded on the control treatment on SCS-1 in the year 2018, followed by the control treatments in the same year on Clark 63 K (44%) and Afgat (41%) (Fig. 5). All the fungicide treatments did not show much differences with each other for yield loss in both years and on all the varieties with (Opera Max 1 l ha<sup>-1</sup>), except Luna Sensation applied on all the varieties i.e., Clark 63K (24%), Afgat (22%) and SCS-1 (20%), in 2017, and Luna Sensation on Clark 63 K (32%), and Nativo on Clark 63 K (28%) in 2017. In the yield loss estimates across locations, the control/unsprayed treatment displayed the highest yield loss of 65% on Clark 63 K, followed by SCS-1 (58%) at Jimma, SCS-1 (55%) at Mettu, and Afgat (51%) at Jimma (Fig. 6). Among the fungicides, Luna Sensation provided the least protection, with the highest yield reductions on all the three varieties i.e., 32% on Clark 63 K at Mettu, and 36% on Clark 63 K, 34% on SCS-1 and 28% on Afgat at Jimma. In addition, the application of Nativo on Clark 63 K provided less protection at Mettu, incurring a yield loss of 28%.

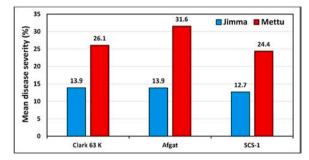
## 3.4. Severity (%) of soybean in response to different varieties and fungicides for the management of ASR across seasons and locations

The severity of ASR was generally higher at Jimma than at Mettu

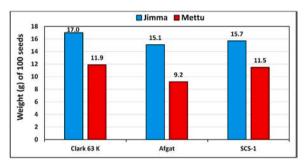
Table 7
Mean value of 100-seeds weight (HSW), severity (%), efficacy (%), area under disease progress curve (AUDPC) in response to the application of fungicides for the management of ASR at Mettu.

| Fungicides                  | $HSW^b$                  |
|-----------------------------|--------------------------|
| Control                     | $8.5\pm0.8b$             |
| Luna Sensation (0.15 lt/ha) | $10.5\pm0.7~\mathrm{ab}$ |
| Nativo (0.5 lt/ha)          | $10.8\pm0.5~ab$          |
| Opera max (0.5 lt/ha)       | $12.0\pm0.9a$            |
| Opera max (0.75 lt/ha)      | $11.0 \pm 0.6$ a         |
| Opera max (1 lt/ha)         | $12.1\pm0.5a$            |
| CV (%) <sup>z</sup>         | 14.7                     |
| Mean                        | 10.8                     |

 $<sup>^{</sup>z}CV$  (%) = coefficient of variation.



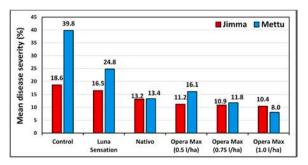
**Fig. 1.** Mean disease severity (%) of Asian soybean rust on three soybean varieties (Clark 63 K, Afgat, and SCS-1) planted in two locations in Ethiopia, Jimma and Mettu, during two years.



**Fig. 2.** Mean weight (g) of 100-seeds of three soybean varieties (Clark 63 K, Afgat, and SCS-1) planted in two locations in Ethiopia, Jimma and Mettu, during two years.

(Fig. 1). The variety Afgat showed the highest severity of 31.6%, while Clark 63 K (26.1%) and SCS-1 (24.4%) showed the next highest severities. At Mettu, all the three varieties displayed nearly the same severities ranging between 12.7% on SCS-1 and 13.9% on Clark 63 K. Among the fungicide treatments, the control showed the highest disease severity of 39.8%, followed by Luna Sensation (24.8%) at Mettu (Fig. 3). The lowest severity of 4% was recorded for the application of Opera Max at the rate of 1 l ha $^{-1}$  at Mettu. The rest of the severities in both locations were nearly similar, with the range of 10.4 for Opera Max (1 l ha $^{-1}$ ) to 18.6% on the control treatment.

Across seasons, the lowest disease severity of 8.0, 8.6 and 9.5% were recorded in response to the application of Opera Max  $(1 \, l \, ha^{-1})$  in 2017 and 2018 seasons, and Nativo  $(0.5 \, l \, ha^{-1})$  in 2017, respectively (Table 9). These treatment combinations were not significantly different with the application of Opera Max  $(0.75 \, l \, ha^{-1})$  in both seasons, Opera Max  $(0.5 \, l \, ha^{-1})$  in 2017, Nativo  $(0.5 \, l \, ha^{-1})$  in 2018 and Luna Sensation  $(0.15 \, l \, ha^{-1})$  in 2017. The highest severity of 39.8% was recorded on the control (unsprayed) treatments in the 2018 season, followed by the



**Fig. 3.** Mean disease severity (%) of Asian soybean rust in response to the application of six fungicide treatments in two locations in Ethiopia, Jimma and Mettu, during two years.

<sup>&</sup>lt;sup>a</sup> Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.

b Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.

**Table 8**The response of soybean varieties across two years (2017 and 2018) to ASR based on severity (%) and area under disease progress curve (AUDPC) at Mettu.

| Years            | Varieties  | Severity (%) <sup>a</sup> | AUDPC                   |
|------------------|------------|---------------------------|-------------------------|
| 2017             | Clark 63 K | $13.6~(21.0)^* \pm 2.0c$  | $758 \pm 102 \text{bc}$ |
|                  | Afgat      | $12.8~(20.6) \pm 1.3c$    | $704 \pm 65 \text{c}$   |
|                  | SCS-1      | $14.2(21.7) \pm 1.8c$     | $767 \pm 93 \text{bc}$  |
| 2018             | Clark 63 K | $19.0(25.0) \pm 2.8b$     | $941 \pm 148b$          |
|                  | Afgat      | $14.2(21.4) \pm 1.8c$     | $721 \pm 95c$           |
|                  | SCS-1      | $23.7(28.1) \pm 3.5a$     | $1,207 \pm 186a$        |
| Mean             |            | 16.3(23.0)                | 850                     |
| CV% <sup>z</sup> |            | 24.2(12.7)                | 24.5                    |

CV (%) $^{\mathbf{z}}$  = coefficient of variation \* Values in bracket are based on square root transformation Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.

Table 9
Mean severity (%), efficacy (%) and area under disease progress curve (AUDPC) values of soybean as affected by fungicide treatments for the control of soybean rust disease across two years (2017 and 2018) at Mettu.

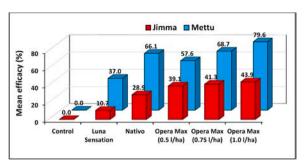
| _  | Severity (%               | b) <sup>a</sup>           | Efficacy  | Efficacy (%)                                  |  |  |
|--|---------------------------|---------------------------|---|---|--|--|
| Fungicides                                     | 2017                      | 2018                      | 2017  | 2018  | 2017   | 2018   |
| Control  | 28.2<br>(32.0)*<br>+ 1.6b | 39.8<br>(39.0)<br>+ 3.6a  | 0 ±<br>0d                                       | 0±0d  | 1,470<br>± 97b                                 | 2,052<br>± 190a  |
| Luna Sensation<br>SC 500<br>$(0.15 1 ha^{-1})$ | 12.9<br>(21.0)<br>± 0.9cd | 24.8<br>(29.6)<br>± 2.7b  | $\begin{array}{l} 52 \pm \\ 5.5 bc \end{array}$ | $\begin{array}{c} 37 \pm \\ 6.0c \end{array}$ | $\begin{array}{c} 713 \pm \\ 53cd \end{array}$ | $\begin{array}{c} 1,232 \\ \pm \ 146 \text{b} \end{array}$ |
| Nativo SC 300<br>(0.5 l ha <sup>-1</sup> )     | 9.5<br>(17.8)<br>± 0.7d   | 13.3<br>(21.1)<br>± 1.7cd | 64 $\pm$ 4.2 ab                                 | 66 $\pm$ 3.8 ab                               | $\begin{array}{c} 520 \pm \\ 45cd \end{array}$ | $668 \pm 89cd$   |
| Opera max<br>(0.5 l ha <sup>-1</sup> )         | 11.6<br>(19.9)<br>± 0.5cd | 16.1<br>(23.4)±<br>1.8c   | 57 ±<br>3.9bc                                   | 57 ±<br>5.4b                                  | $\begin{array}{c} 652 \pm \\ 28cd \end{array}$ | 810 ±<br>95c   |
| Opera max $(0.75  \mathrm{l  ha^{-1}})$        | 10.5<br>(18.8)<br>± 0.8cd | 11.8<br>(19.8)<br>± 1.5cd | 60 ±<br>4.5b                                    | 68 $\pm$ 5.1 ab                               | $598 \pm 41cd$                                 | 577 ±<br>66cd  |
| Opera max (1 l<br>ha <sup>-1</sup> )           | 8.6<br>(17.0)<br>± 0.4d   | 8.0<br>(16.3)<br>± 0.7d   | 68 $\pm$ 2.4 ab                                 | 79 $\pm$ 1.7 ab                               | $\begin{array}{c} 505 \pm \\ 23cd \end{array}$ | 399 +<br>36d   |
| Grand mean<br>CV (%)                           | 16.3(23.0)<br>24.2(12.7)  |                           | 51.0<br>21.2                                    |   | 850.1<br>24.5                                  |  |

<sup>&</sup>lt;sup>z</sup>CV (%) = coefficient of variation \* Values in brackets are based on square root transformation.

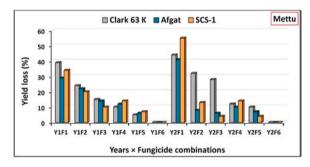
control treatment in 2017 and Luna Sensation (0.15 l  $\rm ha^{-1}$ ) in 2018, which showed severity levels of 28.3 and 24.8%, respectively. When examining varieties alone, SCS-1 had the highest severity (23.7%), followed by Clark 63 K (19%) in 2017, while the rest of the varieties in both seasons did not show significant differences with the lowest severity level (12%) that was recorded on Afgat in 2017 (Table 8).

### 3.5. Efficacy (%) of the fungicides used for the management of ASR across seasons and locations

Generally, higher efficacy of the fungicides was found at Mettu than at Jimma for all the fungicide treatments (Fig. 4). At Jimma, Opera Max  $(1\ l\ ha^{-1})$  gave the highest efficacy of 79.5%, followed by Opera Max 0.75  $l\ ha^{-1}$  (68.7%) and Nativo (66.1%). The lowest efficacy was obtained for Luna Sensation (37.0%), while Opera Max 0.  $l\ ha^{-1}$  gave efficacy of 57.6%. At Jimma, Opera Max at all the three rates gave nearly similar efficacies ranging between 39.1 (0.5  $l\ ha^{-1}$ ) and 43.9% (1  $l\ ha^{-1}$ ). The lowest efficacy (10.7%) was recorded on Luna Sensation, while Nativo gave 28.9% efficacy.



**Fig. 4.** Mean efficacy (%) of five fungicide treatments applied for the control of Asian soybean rust in two locations in Ethiopia, Jimma and Mettu, during two years. Non-sprayed soybean plants served as negative control.



**Fig. 5.** Percent yield loss caused by Asian soybean rust on three soybean varieties (Clark 63 K, Afgat, and SCS-1) treated with two reference fungicides [Luna Sensation (F2) and Nativo SC 300 (F3)], and three rates of Opera Max [0.5 (F4), 0.75 (F5) and  $11 \, \text{ha}^{-1}$  (F6)], during two cropping seasons, 2017 (Y1) and 2018 (Y2), at Mettu, Ethiopia. Losses were calculated using as a reference the yield obtained in the control [unsprayed (F1)] treatment.

The efficacy of all the fungicide applications did not show significant differences, except the control treatments in both years, and the application of Luna Sensation in 2018 (Table 9). The efficacy for the fungicide treatments ranged between 79.5% [Opera Max (1 l ha $^{-1}$ ) in 2018] and 37.0% [Luna Sensation (0.15 l ha $^{-1}$ ) in 2018].

## 3.6. AUDPC value of soybean varieties in response to interaction with fungicides for the management of ASR across seasons and locations

The highest AUDPC value of 1,207 was recorded on SCS-1, followed by Clark 63 K (941) in 2018 (Table 8). The rest of the AUDPC values in 2017 and 2018 did not show significant differences. The lowest AUDPC value of 399 was recorded for Opera Max at 11 ha<sup>-1</sup> in 2018, which did not show significant differences with all the fungicide treatments, except with the application of Opera Max  $(0.5 \, l \, ha^{-1})$  and Luna Sensation  $(0.15 \, l)$ 1 ha<sup>-1</sup>) in 2018 that showed AUDPC values of 810 and 1,232, respectively (Table 9). The highest AUDPC value of 2,052 was recorded on the control treatment in 2018, followed by the control treatment (1,470) in 2017, and Luna Sensation (0.15 l ha<sup>-1</sup>) (1,232) in 2018. At Mettu, the lowest AUDPC values were recorded for all the three rates of Opera Max, and Nativo (0.5 l ha-1) on all the three varieties (Clark 63 K, Afgat and SCS-1), and Luna Sensation on only the variety Afgat (Table 10). The highest AUDPC were obtained for the control treatments on SCS-1 and Clark 63 K, followed by the control treatment on Afgat, and Luna Sensation on Clark 63 K and SCS-1 at Mettu.

### 3.7. Correlation analysis among the studied traits

The correlation analysis revealed strong highly significant positive association of grain yield with efficacy (0.70) and 100-seed weight (0.30) (Fig. 7). Conversely, the grain yield showed strong and highly

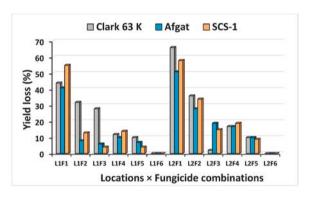
<sup>&</sup>lt;sup>a</sup> Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.

**Table 10**Mean AUDPC of soybean in response to the interaction of varieties and the application of fungicides for the management of ASR at Mettu.

| Fungicide applications  | Clark 63 K <sup>a</sup>   | Afgat   | SCS-1  |
|---|---|---|--|
| Control Luna Sensation (0.15 lt/ha) Nativo (0.5 lt/ha) Opera max (0.5 lt/ha) Opera max (0.75 lt/ha) Opera max (1 lt/ha) | $2,027 \pm 254$ ab<br>$1,183 \pm 300$ cde<br>$494 \pm 56$ ef<br>$835 \pm 198$ def<br>$663 \pm 178$ ef<br>$442 \pm 34$ f | $1,480 \pm 10 bcd$<br>$864 \pm 17 def$<br>$566 \pm 114 ef$<br>$647 \pm 53 ef$<br>$485 \pm 109 ef$<br>$286 \pm 60 f$ | $2,650 \pm 170a$<br>$1,650 \pm 102bc$<br>$945 \pm 142cdef$<br>$946 \pm 208cdef$<br>$585 \pm 34ef$<br>$468 \pm 40f$ |
| CV (%) <sup>z</sup><br>Mean   | 23.4<br>956.9   |   |  |

<sup>&</sup>lt;sup>z</sup> CV (%) = coefficient of variation \*.

<sup>&</sup>lt;sup>a</sup> Duncan's multiple range test at 5% level of significance was used to declare significant differences among means, values sharing the same letter are not statistically different.



**Fig. 6.** Estimated yield loss (%) caused by Asian soybean rust on three soybean varieties (Clarck 63 K, Afgat and SCS-1) treated with the control [unsprayed (F1)], two reference fungicides [Luna Sensation (F2) and Nativo SC 300 (F3)], and three rates of Opera Max  $[0.5 \ l\ ha^{-1}\ (F4),\ 0.75\ l\ ha^{-1}\ (F5),\ and\ 1\ l\ ha^{-1}$  (F6)], in two locations, Mettu (L1) and Jimma (L2), in the 2018 cropping season.

significant negative association with yield loss, severity and AUDPC with correlation coefficients of (-0.96), (-0.56), and (-0.50). A strong and highly significant correlation was found between 100-seed weight and grain yield (0.30). The association of 100-seed weight was strong and highly significant negative with severity and AUDPC with respective correlation coefficients of -0.41 and -0.48. Yield loss has showed strong, highly significant and positive association with severity (0.55), and AUDPV (0.48), and strong, highly significant and negative association with efficacy (-0.72). The association between severity and efficacy (-0.59), and efficacy and AUDPC (-0.49) was strong, highly significant negative, while severity and AUDPC showed a strong highly significant positive correlation of 0.99.

### 4. Discussion

The current study evaluated three soybean varieties protected with five fungicide treatments, at two locations, for two years, for the control of ASR, a devastating disease. Results from this study indicated that Opera Max, a fungicide recommended for the control of ASR in Ethiopia, at both rates of  $11\,\mathrm{ha}^{-1}$  and  $0.75\,\mathrm{l\,ha}^{-1}$  on all the varieties, locations and seasons provided equivalent and the highest levels of protection. The ANOVA for the individual locations revealed significant variety  $\times$  fungicides interactions for only, AUDPC at Mettu. This indicates the development of ASR disease is affected by the interaction of varieties and fungicides. The fact that each of the varieties and fungicides showed highly significant differences for most of the studied traits indicates the importance of varieties and fungicides in the management of ASR disease independently. The highly significant interactions of varieties,

fungicides and seasons, and varieties, fungicides and locations for grain yield, and two-way interactions of varieties and fungicides, and varieties and seasons for AUDPC indicate the important role of the combined effects of the genetics of varietal resistance, fungicides treatment and seasonal factors in slowing down the development and aggressiveness of ASR. Selecting the right variety with better resistance to ASR, coupled with an effective fungicide, needs to be part of the disease management practices. In line with this, Hoffmann et al. (2019) reported the use of cultivars with good ASR. The highly significant differences in fungicide treatments for all the studied parameters will help identify the right type and rate of fungicide for ASR control. Sautua et al. (2020) also emphasized the importance of the type of fungicide, the degree of susceptibility of the variety, and environmental variables in controlling stem rust in wheat.

Interestingly, the application of Opera Max at the rate of  $11 \, \mathrm{ha}^{-1}$  and 0.75 l ha<sup>-1</sup> gave the highest yield on all the three varieties and across both seasons. This shows that the two rates of Opera Max provided comparable control of ASR. The application of Opera Max (0.5 l ha<sup>-1</sup>), Nativo and Luna Sensation gave a yield level that was not statistically different from each other in 2017, showing the relatively similar level of disease control provided by these fungicides. The rest of the treatments i. e., Opera Max (0.5 l ha<sup>-1</sup>), Luna Sensation and Nativo showed a similar response in the control of soybean rust in all the varieties in 2017. However, in 2018 Opera Max (0.5 l ha<sup>-1</sup>) on Clark 63 K and Afgat; Nativo on Afgat and SCS-1 and Luna Sensation on Afgat gave similar (P > 0.05) protection with the fungicide treatment [Opera Max (1 l ha<sup>-1</sup>)] that showed the highest yield response. In 2017, Luna Sensation did not produce a better yield than the control treatment on all the varieties, while in 2018 both Luna Sensation and Nativo on Clark 63 K did not produce a better yield than the control treatment. Across locations, all the three Opera Max rates at Mettu, and all the Opera Max rates, except Opera Max (0.5 l ha<sup>-1</sup>) on SCS-1 at Jimma produced the highest grain yield that did not differ with each other, including the variety that produced the maximum yield i.e., Afgat at Jimma (3.5 t  $ha^{-1}$ ). On the other hand, Luna Sensation applied on all the varieties at Jimma, and on Clark 63 K at Mettu showed non-significant differences with the control. Similarly, Nativo applied on Clark 63 K at Jimma, and SCS-1 at Mettu did not significantly differ for grain yield from the control. This indicates that Opera Max at both 0.75 and  $1\,\mathrm{l\,ha^{-1}}$  rates gave better protection to ASR. The chemicals used for comparison i.e., Luna Sensation and Nativo did not provide reasonable control of the disease at the current rate of applications.

The yield loss on the control treatments ranged between 29 and 65% across seasons, locations and varieties, indicating the very high yield losses caused by the disease in the study areas. Across seasons, the highest yield losses were recorded on all the three varieties i.e., Afgat (55%), Clark 63 K (44%), and SCS-1 (41%) on the control treatment in 2018, whereas across locations the highest yield loss of 65% was recorded on Clark 63 K, followed by SCS-1 (58%) at Jimma on the control treatment. The highest yield loss recorded in this study (65%) might be regarded as very high relative to the 40% yield loss reported in Japan (Bromfield, 1984), and moderate relative to yield losses of 60%-100% reported in Brazil, Paraguay, and South Africa in the early 2000s (Yorinori et al., 2005). The yield reductions due to incomplete protection of Opera Max at all the rates and on all the varieties in both 2017 and 2018 did not show much difference from each other. This indicates that Opera Max provided comparable protection for ASR, irrespective of the application rates. In contrast, the application of Luna Sensation on all the three varieties at Jimma and on Clark 63 K at Mettu showed a relatively high yield reduction. The application of Nativo on Clark 63 K also displayed a relatively high yield loss. This gives another evidence that the use of both Luna Sensation and Nativo at their current rate did not provide adequate protection against ASR.

The control treatment in 2018 showed the highest severity and AUDPC values, followed by the control in 2017 and Luna Sensation in 2018. Across seasons, all the Opera Max rates and Nativo applications in

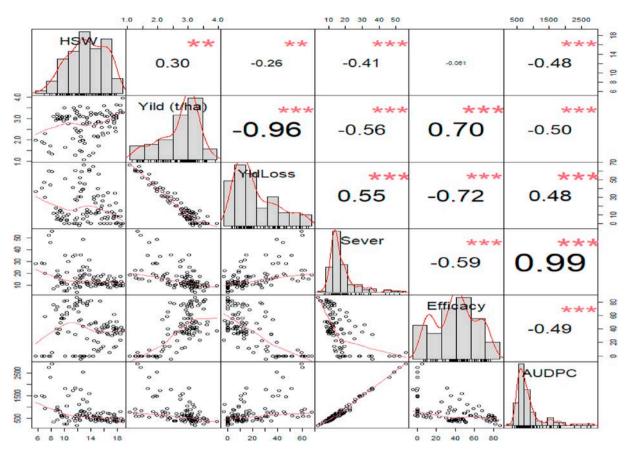


Fig. 7. Correlation analysis among the studied traits in response to varieties and fungicides and their interaction for the management of Asian Soybean Rust disease across seasons and locations.

both 2017 and 2018, except Opera Max  $(0.5\,l\,ha^{-1})$  in 2018 showed the lowest disease severity values. Across locations, the application of Opera Max  $(1\,l\,ha^{-1}$  and  $0.75\,l\,ha^{-1})$  at both Mettu and Jimma, and Opera Max  $0.5\,l\,ha^{-1}$  only at Jimma showed the lowest severity level. In line with this Delaney et al. (2018) also reported significantly reduced severity of ASR, following fungicide treatments. The control treatment at Mettu showed the highest level of severity, followed by Luna Sensation at Mettu.

At Jimma, the lowest AUDPC values were obtained for all the fungicide applications on all the varieties. At Metu location, the spraying of Opera Max (1 l ha<sup>-1</sup> and 0.75 l ha<sup>-1</sup>) on all the varieties, Opera Max (0.5 l ha<sup>-1</sup>) on Afgat, and Nativo on Clark 63 K and Afgat gave the lowest AUDPC values, and did not significantly differ from each other. This may also indicate that Opera Max in the three different rates might be alternatively used to control ASR. Results from the current study suggest that the Luna Sensation rate used, and recommended by the manufacturer, are not sufficient for the management of ASR in Ethiopia, as it gave AUDPC value that is not significantly different from the control (unsprayed) treatment. Even though no literature was found on the use of Luna Sensation for the control of ASR, Ravikumar et al. (2020) reported the best control of purple blotch disease of onion using Luna Sensation at the rate of 600 ml ha<sup>-1</sup>, which might be another evidence that the rate of Luna Sensation for the control of ASR was not optimal. Hence, further studies need to be conducted to revise the rate of application of this fungicide. Certainly, it should be investigated whether using higher rates of Luna Sensation do not pose adverse effects to workers, consumers, and micro and macro-organisms.

Across seasons, the efficacy of all fungicides, except Luna Sensation, did not show significant differences from each other. At the same time, Opera Max  $1\,l\,ha^{-1}$  produced the highest efficacy at Mettu, followed by Opera Max  $0.75\,l\,ha^{-1}$ , Nativo SC, and Opera Max  $(0.5\,l\,ha^{-1})$  at Mettu.

In line with this Juliatti et al. (2017) also reported the effective use of Nativo  $(0.5 \, l \, ha^{-1})$  and Opera Max  $0.5 \, (l \, ha^{-1})$  for the control of ASR in Brazil. Both Opera Max and Nativo SC 500 are mixtures of the quinone outside inhibitors (QoI) (strobilurins), and demethylation inhibitors (triazoles), and combines the protectant and curative properties of the two groups of fungicides. Hence, both fungicides can successfully prevent penetration and establishment of the pathogen and progression of the disease after establishment (Juliatti et al., 2017; Tenuta et al., 2008; Yorinori et al., 2005). The effective use of a mixture of the two groups of fungicides for the control of ASR was reported by several workers (Juliatti et al., 2017; Mueller et al., 2009; Tenuta et al., 2008; Yorinori et al., 2005). The findings of this study generally indicate that all the three varieties showed susceptibility to ASR; however, Afgat showed low relative susceptibility to ASR, compared to SCS-1 and Clark 63 K; whereas SCS-1 relatively displayed the highest susceptibility. Hence, considering the low genetic resistance of the released varieties under production and the high yield losses the disease may cause, applying chemical fungicides to control ASR is justifiable in areas where the disease is commonly occurring in the country. In line with this, Mueller et al. (2009) also underlined the enormous yield losses the disease may cause if it is not managed with timely applied fungicides. Godoy et al. (2016) also reported intensified use of fungicides for the control of ASR due to the resurgence of the disease and lack of varieties with a good resistance level.

The application of Opera Max  $(1 l ha^{-1})$  treatment produced the highest 100-seed weight, followed by Opera Max  $(0.75 l ha^{-1})$ , and  $0.5 l ha^{-1}$ ) and Nativo SC, which was not statistically different from each other (Table 8). The control treatment produced the lowest 100-seed weight, followed by the application of Luna Sensation. The findings of Delaney et al. (2018) also reported a significant increase of 100 seed weight and yield in response to fungicide applications. It is evident from

these findings that one of the mechanisms the rust disease causing yield reduction is through reducing seed weight and size, also indicating treating soybean rust infested fields with fungicides can improve seed size, and thereby, grain yield. Sharma et al. (2016) studied the effect of three fungicides, including Opera Max, to control stripe rust in wheat and reported that all the fungicides controlled the disease, increased grain yield, and thousand kernel weight.

The findings of this study proven Opera Max at the rate of 1 and 0.75 l ha $^{-1}$  effectively reduced the severity of ASR and sufficiently justified the need to use this fungicide for the control of ASR. However, excessive use of fungicides needs to be avoided; as reported by Langenbach et al. (2016) it might result in the appearance of new fungal races insensitive to the fungicides in use. Hence, we recommend using 0.75 l ha $^{-1}$  of Opera Max for the control of ASR, as it consistently provided equivalent protection of the ASR with the highest rate (1 l ha-1) of Opera max fungicide. The efficacy of the chemicals was generally low at Jimma, compared to the relative efficacy at Mettu, which might be due to the agro-climatic differences between the two locations. In line with this, Mengesha (2020) underlined the importance of the interaction of varieties, fungicides, along with environmental variables for the development of stem rust disease in wheat.

The correlation analysis revealed strong and highly significant negative association of grain yield with yield loss, severity, and AUDPC, which confirmed the high level of impact of the disease on the productivity of the soybean. The strong highly significant positive association of efficacy with grain yield and the strong highly significant negative association of severity and efficacy and efficacy and AUDPC indicates the important role of fungicides applications for the management of Asian soybean rust disease and in improving the productivity of soybean. A positive and highly significant association of 100-seed weight with grain yield affirms seed size as a very important yield component trait. Similar to grain yield, 100-seed weight showed a strong, highly significant negative correlation with severity and AUDPC, indicating seed size was also highly affected by the Asian soybean rust disease, which consequently contributed to the reduced productivity of the crop. The strong, highly significant positive association between severity and AUDPC was expected as AUDPC is the product of disease severity.

### 5. Conclusion

In general, the results of this study provided strong evidence that the use of fungicides to control ASR in areas where the disease is prevalent is important considering the existing susceptibility of the soybean varieties under production to prevent the high yield loss that reached up to 65%. This study generally showed that Luna Sensation at the rate of 0.15 l ha<sup>-1</sup> may not provide effective control of ASR. Opera Max at the rate of 0.75 l ha<sup>-1</sup> provided efficient and equivalent protection with a 1 l ha<sup>-1</sup> rate of the fungicide. Hence, Opera Max 0.75 l ha<sup>-1</sup> is recommended for the control of ASR, even if the maximum protection for the disease was obtained from the 1 l ha<sup>-1</sup> rate. Even though the evidence was not strong, the application of Opera Max at the rate of 0.5 l ha<sup>-1</sup> and Nativo (0.5 l ha<sup>-1</sup>) provided good protection against the soybean rust disease. The results from this study should be communicated to farmers and stakeholders of the soybean value chain for the appropriate use of fungicides and varieties to enhance the productivity of soybean in Ethiopia and other countries with heavy occurrence of the disease to reduce yield losses caused by ASR. Although on-station trials, results of the current study demonstrate that soybean productivity in Ethiopia can reach yields comparable to averages in other countries where the crop is intensively cultivated, in order to attain similar yields, growers would have to effectively control ASR through use of appropriate integrated disease management packages.

#### **Author's contributions**

Abush Tesfaye Abebe: Conceptualization, Methodology, Data analysis, Writing- Original draft preparation, Reviewing and Editing, Kifle Belachew: Methodology, Data Collection, Writing- Reviewing and Editing, Mesfin Hailemariam: Data Collection, Writing- Reviewing and Editing, Yechalew Sileshi: Data Collection, Writing- Reviewing and Editing, Alejandro Ortega-Beltran: Supervision. Writing- Reviewing and Editing.

### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix 1. Product label for Opera max

http://www.royalagroscience.com/en/basf5.

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