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# **Diallel analysis of scald and net blotch resistance in barley (***Hordeum vulgare* **L.)**

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**Scald and net blotch are major foliar diseases of barley causing high yield losses worldwide including Ethiopia. Development of varieties with double resistance is an effective approach of managing both diseases. However, the genetic background of the barley parents was not studied for future resistance breeding. Thus, the objective of the study was to assess genotype performances against scald and net blotch, investigate gene effects involved in controlling the diseases for future breeding and suggest better breeding system. Therefore, twenty eight barley genotypes were evaluated in a randomized complete block design with three replications at Holetta in 2015. Combining ability analysis showed general combining ability (GCA) and specific combining ability (SCA) was highly significant (P0.01) for initial disease severity, final percent severity and Area under disease pressure curve (AUDPC) for both scald and net blotch except for SCA in initial and AUDPC of net blotch. This revealed the importance of additive and non-additive gene actions in controlling resistance for both diseases. HB1307 and HB42 parents were general combiner for scald and net blotch resistance. The result suggests the possibility of developing diverse populations from superior GCA parents to scald and net blotch through diallel intermating of selected segregants followed by selection at late generations. And final disease rating can be useful for evaluating a large number of barley genotypes to both diseases.**

**Key words:** Barley (*Hordeum vulgare* L.), additive and non additive gene effects, combining ability.

# **INTRODUCTION**

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world after maize, rice and wheat in production and its world average productivity was about 3.0 tons ha<sup>-1</sup>while in some top producing countries exceeds 5 tons ha<sup>-1</sup> (FAOSTAT,  $2016$ ). It is among the

first domesticated cereal crops in Ethiopia and its utilization is deep rooted in Ethiopian tradition. Landraces are diverse and source of resistance to several barley leaf diseases (IBC, 2008). Barley is widely cultivated predominantly between altitudes of 2000 and 3000

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m.a.s.l. in Ethiopia (Berhane et al., 1996). However, the widespread occurrences of barley scald and net blotch foliar diseases is limiting barley production worldwide including Ethiopia (Tekauz 2003; Xi et al., 2008). Significant grain yield and quality losses has been occurred in Australia on barley due to net blotch (Stepanović et al., 2016) and losses of up to 50% of yield with possible complete loss depending on susceptible cultivar and environmental conditions was reported due to net blotch (Steffenson et al., 1996). On the other hand, worldwide scald disease could cause about 100% losses on susceptible cultivars under severe epidemics (Yahyaoui, 2004) and yield decreases of up to 40% and reduced grain quality has been also reported due to scald (Zhan et al., 2008).

Furthermore, barley net blotch and scald diseases are widely distributed foliar diseases of barley limiting its production in Ethiopia. High yield loss reaching up to 34% has been reported due to net blotch (Yitbarek et al., 1996). Yield losses reaching about 67% have been recorded due to scald in Ethiopia (Yitbarek et al., 1998). The yield loss assessment over locations in central high lands of Ethiopia showed mean grain yield loss due to net blotch and scald combined ranged from 14 to 25% in 1999 and 2000 years, respectively while yield losses of 9.8 to 31.54% resulted from scald in western Ethiopia (Meki and Asnakech, 2004).

Research study reports on various *Rynchosporium cummune* isolates collected from different agro-ecologies of Ethiopia indicated divergence in phenotypic and virulence (Kiros et al., 2004) and high genetic diversity in the pathogen population and high gene flow between regions and among populations in Ethiopia (Kiros, 2004) indicating the presence of high pathogen variability which fastens resistance of cultivars break down unless cultivars with multiples resistance genes are developed against scald and net blotch. In addition, the use of few cultivars for long period of time would result in an increase of disease epidemics causing high yield loss. The deployment of resistance suggested as an important and successful component in preventing and controlling diseases that is relatively inexpensive, biologically safe and convenient for the farmer. It can be used as a component of integrated disease management programs (Hogenboom, 1993). Moreover the use of cultivars with double resistance is the most effective method in controlling both diseases (Cherif et al., 2007). In breeding of high yielding cultivars with desirable traits, success depends on selection of suitable parents and appropriate breeding method. In a hybridization program, parents should be chosen on the basis of their combining ability. Hence the diallel mating design provides breeders useful genetic information on combining ability to help them devise appropriate breeding (Bertan et al., 2014) and selection strategies (Griffing, 1956; Zhang et al., 2005).

Combining ability analysis provides the basis for selecting good combiners and also for understanding the

nature of gene action (Rajendran et al., 2014). Research on barley showed that resistance to net blotch was controlled by either one or several genes (Steffenson et al., 1996; Williams et al., 2003) and as monogenic (Douiyssi et al., 1996). In another barley crosses, net blotch resistance was conditioned by high additive and non-additive gene effects with high partial resistance reaction (Arabi et al., 1990).

In Ethiopia, in variety development every year landrace and exotic germplasms had been evaluated for desirable agronomic traits including for scald and net blotch diseases but most of them were found susceptible to scald, net blotch so that few entries are advanced for further study and there was a limited success in screening and hybridization activities; but it was not adequate so that yet breeding for resistance to scald and net blotch disease is considered as a future research focus (Birhanu et al., 2005; Bayeh and Berhane, 2011). Therefore, owing to few resistant cultivars under use for long period of time and an increasing pressure due to existing pathogen variability in the region, breeding for cultivars that combine different resistant genes to the barley foliar diseases is very important. In the present study, seven barley cultivars having different levels of resistance to net blotch and scald barley diseases were included and these nature of resistance genes contained in these cultivars were not studied. For successful breeding for resistant barley cultivars or plants, the knowledge on the genetic background of the parents and the suitability of cultivars for hybridization program should be investigated. Hence, this study was conducted with the objectives of estimating combining ability variances and gene actions controlling the inheritance of scald and net blotch resistance genes of barley for future breeding.

## **MATERIALS AND METHODS**

## **Experimental Site**

The study was conducted in 2015 main cropping season at Holetta Agricultural Research Center, which is about 30 km west of Addis Ababa. The area is located at an altitude of 2390 m above sea level (m.a.s.l), latitude of 09°04' N and longitude of 38° 30'E (http://www.eiar.gov.et). Holetta is one of hot spot area for scald and net blotch barley disease where most of screening has been done in Ethiopia.

## **Planting materials and experimental design**

Seven barley cultivars (Table 1) with different levels of resistance and susceptibility to scald and net blotch diseases and origin were used in half diallel crossing in 2014/2015 main cropping season to generate 21  $F_1$  crosses for field evaluation. The  $F_1$  crosses were obtained by hand emasculation and pollination in the field. Then a total of twenty eight genotypes including seven parents and 21  $F_1$ crosses were planted at Holetta in a randomized complete block design with three replications during the 2015 main cropping season. Seeds of each genotype were sown in two rows of 2.5 m

S/N	<b>Cultivars</b>	Year of registration/release	Tvpe of barley	<b>Row</b> number	Origin/history	Scald and net blotch <b>reaction</b>
	Sabini <sup>'</sup>	2011	Malt	Two	Introduction	Susceptible
	Grace <sup>'</sup>	2013	Malt	Two	Introduction	Susceptible
	Misrach <sup>D</sup>	1998	Food	<b>Six</b>	Pure line selection from Acc. Kulumsa 1/88	Moderately resistant
4	HB1307 <sup>H</sup>	2006	Food	<b>Six</b>	A cross made from Awura gebs-1/IBON93/91, EH-1700/F <sub>71</sub> .B <sub>1</sub> .63	Resistant
	Miscal-21 <sup>H</sup>	2006	Malt	Two	Introduction from ICARDA/CIMMYT and developed by Holetta	Moderately resistant
	HB42 <sup>9</sup>	1985	Food	<b>Six</b>	Developed by exotic x landrace IAR/ H/81/compound 29//compound 1420/cost	Highly resistant
	Agegnehu <sup>SR</sup>	2007	Food	Six	Pure line selection from Acc.218950-08	Moderately resistant

**Table 1.** Description of seven barley cultivars used in half diallel crossing in 2014/5.

<sup>1</sup>ntroduced (personal communication with Dr.Berhane Lakew),<sup>D</sup>Developed by Holetta Agricultural Research Center and released by Debre Berhan Agricultural Research Center, BReleased and developed by Holetta Agricultural Research Center, <sup>SR</sup>Released and developed by Sirinka Agricultural Research Center. Sources: (Berhane and Alemayehu, 2011; Wosene et al., 2015).

length and 0.40 m width at 15 cm between plants.

To increase the disease epidemics, the spreader rows of scald susceptible variety, Sabini, was planted surrounding each block and plot.

## **Scald and net blotch assessment**

Scald and net blotch disease severity was scored on ten randomly selected plants in each plot using double digit scale (D1D2, 00-99) based on Saari and Prescott (1975) in the field under natural condition. The first digit (D1) indicates vertical disease progress on the plant and while the second digit (D2) refers to severity measured as diseased leaf area. Disease scoring was started on 10 September 2015 at 53 growth stage (Zadoks et al., 1974) as modified by Tottman and Makepeace (1979) and repeated five times at seven days interval.

The plot mean severity scores of each plot was converted into percent severity scale for all the growth stages such that for each score, the percentage of disease severity was estimated using the formula of disease severity (DS) (%) = (D1/9)  $\times$  (D2/9)  $\times$  100 (Sharma and Duveiller, 2007). Area under disease pressure curve (AUDPC) was calculated to estimate the scald and net blotch severity over time based on the five periods of record of percent disease severity estimations according to In and 0.40 m with at 15 cm between plants.<br>
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AUDPC = \sum_{i}^{n-1} \left[ (Y_i + Y_{i+1})/2 \right] (T_{i+1} - T_i)
$$

where Y<sub>i</sub>=the disease severity on the i<sup>th</sup> date,  $T_{(i+1)}$ - $T_{i=1}$  time or days between two disease scores, n=number of dates on which the disease was recorded.

#### **Data analysis**

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure in the SAS version 9.1software (SAS, 2008). Then diallel analyses were conducted according to Griffing's method 2 and model 1(fixed effect) (Griffing, 1956) using the SAS program for Griffing's diallel analysis by Diallel SAS program of Zhang et al. (2005). The broad and narrow sense heritability was calculated following Griffing (1956). Baker ratio was determined according to Baker (1978) and graphs were prepared by Minitab software version 17 (Table 1).

## **RESULTS AND DISCUSSION**

## **Mean performance of barley genotypes to scald and net blotch diseases**

The analysis of variance for initial percent severity

(except for net blotch,  $P\leq 0.05$ ), final percent severity and AUDPC for scald and net blotch showed highly significant  $(P \le 0.01)$  genotypic differences (Table 2). This indicates the presence of wide genetic variation in response to scald and net blotch diseases which makes suitability for selection. And the mean performance estimates of the genotypes varied for initial percent severity, final percent severity and for AUDPC of scald and net blotch (Figure 1).

As compared to net blotch, the scald severity symptom is high showing faster epidemic development may be because of the polycyclic nature of scald disease. Considering the AUDPC estimates for scald, it appears that Sabini x HB42, Grace x HB42, Misrach x HB42, HB1307 x HB42, HB42 x Agegnehu and Miscal-21 x HB42 crosses and HB42 parent showed increased scald resistance response While Grace x Misrach, Grace x HB1307, Grace x Miscal-21, HB1307, HB42 and Miscal-21 showed increased net blotch resistance (Figure 1). However, from previous performance history, Grace is susceptible parent to net blotch and scald but as opposed to that in



**Table 2.** Analyses of variance for percent severity of scald and net blotch as well as AUDPC in 2014/15 cropping season.

\*,\*\* significant at the 0.05 and 0.01 probability level, respectively., ns=non significant, DF-degree of freedom, GCA=general combining ability; SCA=specific combining ability, AUDPC-<br>area under disease progress curve, CV=



**Figure 1.** Mean performance of 28 barley genotypes for initial percent severity, final percent severity and AUDPC for scald and net blotch diseases at Holetta in 2015.



**Figure 2.** A regression line drawn using total disease severity (%) of scald and net blotch on 28 barley genotypes assessed over five weeks at Holetta, Ethiopia, in 2015.

this study the data showed as if it was resistant to net blotch however, this was mainly due to rapid infestation of barley leaf part by scald than net blotch. With multiple severity readings, the AUDPC is useful as a measure of slow rusting resistance (Wilcoxson et al., 1974) and AUDPC which shows both severity and rate of disease development (Shaner and Finney, 1977). The AUDPC can be useful to assess quantitative disease resistance (Jeger and Viljanen-Rollinson, 2001). From the regression line (Figure 2) drawn for both scald and net blotch showed that the rate of disease development for scald was very fast and linearly progressing over time than net blotch disease. This could be attributed to the polycyclic nature of scald pathogens. It clearly shows the significance of the scald disease as compared to net blotch in prioritizing. However, it needs further confirmation by testing under controlled environment separately as the interaction among the pathogens of both diseases may affect the severity. The disease development was peak at the final scoring period for both diseases at Holetta area so that the final disease rating can be useful for screening a large number of barley genotypes to save time and resource.

## **Combining ability analysis for scald and net blotch**

There was highly significant ( $P \le 0.01$ ) general combining ability (GCA) and specific combining ability (SCA) for initial percent scald severity; final percent scald severity and AUDPC (as % days) for scald (Table 2).This revealed the high involvement of additive and nonadditive gene effects in controlling scald resistance. In addition, the Baker ratio and narrow sense heritability was high for final percent severity for scald, but average for initial percent severity and AUDPC for scald (Table 2) which showed the proportional influence of additive gene effects and non-additive gene effects on controlling the inheritance of genes for increased resistance. High genetic advances could be realized when working on traits with higher additive genetic variance (Baker, 1978).

Lesser broad sense heritability (0.64) estimates for scald resistance to all scald isolates than this study was reported (Feriani et al., 2012) but similarly high broad sense heritability of 0.850 and 0.967 was obtained in two barley crosses (Aoki et al., 2011) for scald resistance. In the study of  $BC<sub>7</sub>$  generation of barley, scald resistance alleles were mainly allelic or additive in the near isogenic

lines (NILs) and GCA effects were much stronger than SCA (Patil et al., 2002). Another research indicated that resistance genes to scald in barley are governed by both 'major' and smaller 'minor' genes that generally has additive effects (Zhan et al., 2008). From five scald resistance genes identified on four Ethiopian barley cultivars showed that three genes were dominant in action and two were recessive (Segenet, 1984). Similar investigation on inheritance of scald resistance on barley lines generated from resistant and susceptible cultivars on  $F_1$ ,  $F_2$ ,  $F_{4:5}$  recombinant inbred lines (RIL) showed a single dominant gene for resistance (Singh et al., 2003). Additive variance is a measure of additive gene action and this gene action is the measure cause of resemblance between relatives and progress by selection is directly proportional to the degree of resemblance between the parent and its progeny (Manickavelu et al., 2006).

Parents and/or crosses with negative values of GCA effects and SCA effects are important to select the most resistant genotypes. GCA and SCA effects for initial percent scald severity, final percent scald severity and AUDPC for scald are shown in Table 3. Combining ability provides the basis for selecting good combiners and also for understanding the nature of gene action (Rajendran et al., 2014) and a parent with a significant negative GCA value would contribute a high level of disease resistance and whereas a parent with a positive value would contribute a high level of susceptibility (Hakizimana et al., 2004). Thus, HB42 and HB1307 parents showed highly significant negative GCA effects for initial percent severity, final percent severity and AUDPC for scald indicating they are the best general combiners for scald resistance including Miscal-21 for initial percent severity and AUDPC for scald.

Therefore, the genotypes may contain vertical resistance which is effective against initial innoculum and there were also genotypes that remained restricting the disease development without significant changes from the initial to final severity symptom equivalent to horizontal or quantitative resistance according to Van der plank (1984). Furthermore, comparison between HB42 and HB1307 with GCA effects in the same direction showed that GCA effects of both HB42 and HB1307 parents were highly significantly different from each other at 1% level suggesting that HB42 was superior general combiner for increasing scald resistance.

In exploiting heterosis, the usefulness of a particular cross is judged by the SCA effect of component cultivars and hybrids are evaluated depending on their SCA effect. Thus, the SCA effects for the 21 barley hybrids for initial percent scald severity, final percent scald severity and AUDPC as percent of days for scald is indicated in Table 3. From 21 crosses, about ten crosses (47.6%), twelve (57%) and nine (47.6%) crosses showed negative SCA effects for initial percent scald severity, final percent scald severity and AUDPC as percent for scald, respectively,

showing wide involvement of non-additive genes in reducing disease symptom and increasing resistance to scald. The negative and significant SCA effect in some hybrids for AUDPC to scald indicated slowing of scald disease epidemics. Whereas in the case of net blotch, the GCA effect showed highly significant  $(P \le 0.01)$ variation for initial percent severity, final percent severity and AUDPC while SCA effect was highly significant for final percent severity (Table 2). This indicated the predominance of additive to non-additive gene effects in controlling resistance to net blotch. Hence, the best net blotch resistant can be produced by crossing two barley parents with the lowest symptom rating GCA effects.

On the other hand the Baker ratio and narrow sense heritability was medium for initial percent severity, final percent severity and AUDPC for net blotch (Table 2) indicating both additive gene effects and non-additive gene effects would have equal influence in controlling the inheritance of increasing resistance. The exhibition of high additive and non-additive genetic effect to net blotch resistance was also reported by Arabi et al. (1990). In another finding, average effects of alleles showed greater importance than dominance in controlling resistance to net blotch (Douglas and Gordon, 1985) and it was also indicated that resistance genes controlling net blotch was inherited monogenetically on barley (Douiyssi et al., 1996).

Besides this, O'Boyle (2009) reported net type net blotch resistance were controlled by single dominant genes. Another study on the genetics of resistance of  $F_1$ ,  $F<sub>2</sub>$  and doubled-haploid barley lines to three isolates of net blotch showed that resistance was controlled by one recessive gene, either one dominant gene or two complementary genes to three recessive genes in each cultivar (Ho et al., 1996). Similarly closer estimated values to this study was indicated on broad-sense heritability estimates ranging from 0.72 to 0.85 for the different disease parameters investigated in barley (Cherif et al., 2010). While lesser estimates than this study was observed in earlier report for broad and narrow sense heritability levels (mostly 40 to 60%) for net blotch resistance in barley (Douglas and Gordon, 1985).

As Ribeiro do vale et al. (2001) extensively reviewed on the genetic of resistance in most of crops, resistance is most often controlled by major genes which are often inherited dominantly, less frequently recessively. Major resistance genes often occur in a surprisingly high numbers and many major resistance genes operate in a gene-for gene way. And minor or polygenic inheritance of resistance has been reported as well, but its much lower frequency is most likely due to the more difficult nature of the research than to a truly lower frequency. And the expression of resistance genes can be modified by the action of other genes (epistasis), the development stage of the plant or the environment.

In selection of the most resistant genotypes, those with negative GCA and SCA estimates are useful to consider



Table 3. Mean performances, GCA of parents and SCA effects of F<sub>1</sub> hybrids for scald and net blotch disease severities and AUDPC on barley.

\*, \*\* significant at the 0.05 and 0.01 probability level, respectively, ns=non significance, Means in the same column with the same letter are not significantly different, S.E(gi) -standard error for all GCA, S.E(<sub>gi-gi</sub>) -standard error for testing the significance among two superior significant GCA effects, S.E(<sub>Sij</sub>)-standard error for testing all SCA. MeanSc=mean of scald, MeanNB=mean of net blotch values, GCASc=GCA of scald, GCANB=GCA of net blotch.

in resistance breeding. Genotypes with negative values of are useful to consider. In this study, Miscal-21 and Agegnehu showed negative and highly significant GCA effect for final percent severity and AUDPC for net blotch indicating that they are best general combiners for net blotch disease resistance. HB1307 also exhibited negative GCA to reduce disease severity symptom and increasing resistance to net blotch disease (Table 3). Overall, HB1307 and HB42 barley parents were good general combiners for resistance to net blotch and scald diseases.

Hybrids are predicted from their SCA effects. From 21 crosses obtained from this diallel cross, 12 hybrids (57%) showed negative SCA effects for final percent severity of net blotch (Table 3). Grace x HB1307 showed significant negative SCA effect for final percent severity. While for AUPDC of net blotch, nine crosses (42.9%) had negative SCA effects and Grace x Misrach hybrid showed significant negative SCA effect indicates high contribution of non-additive genes in reducing disease symptoms. The cross combination of the two susceptible cultivars with the resistant parent HB1307 resulted progenies with increased resistance. Barley cultivars and barley lines introduced from abroad for research or commercial purpose like Sabini and Grace were commonly observed susceptible to net blotch and scald in Ethiopia. Therefore, HB1307 and HB42 can be useful sources of resistance genes in breeding and improving of the resistance of susceptible and low yielding introduced and/or lines which otherwise are agronomically desirable via different breeding systems.

In conclusion, barley genotypes evaluated showed high genetic variability for scald and net blotch diseases revealing the possibility of effective selection. GCA and SCA effects were high significant among 28 barley genotypes (7 parents + 21 crosses) for the initial, final percent severity and AUDPC for scald and net blotch diseases except the initial and AUDPC of net blotch.

Generally additive and non-additive types of gene effects were involved in governing the inheritance of reducing disease symptom and increasing resistance for both diseases. Both HB42 and HB1307 parents with highly significant and negative GCA effects can be good sources of resistance genes to scald and net blotch. The final disease rating period can be useful for evaluating a large number of barley genotypes to both diseases to save time and resources. Thus the finding suggests that there was wide potential to develop scald and net blotch resistant barley lines. Hence, the resistant additive alleles found in the resistant parents can be fixed using diallel selective mating of segregants followed by selection at later generations.

# **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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