Hindawi
Advances in Agriculture
Volume 2023, Article ID 1553452, 9 pages
https://doi.org/10.1155/2023/1553452

Research Article

Identification of Host Critical Stage Affected by *Orobanche crenata* and Variation in the Resistance of Faba Bean Genotypes under Infested Field and Controlled Conditions in Ethiopia

Lemma Diriba

Ethiopian Institute of Agricultural Research, Mehoni Agricultural Research Center, P.O. Box 47, Mehoni, Tigray, Ethiopia

Correspondence should be addressed to Lemma Diriba; lemmadiriba@gmail.com

Received 20 May 2022; Revised 14 October 2022; Accepted 19 January 2023; Published 10 February 2023

Academic Editor: Amelia Salimonti

Orobanche crenata is a serious parasitic weed and a major constraint on legume crops, particularly for faba bean, which causes about 75–100% of yield losses in Ethiopia. Twenty faba bean genotypes were evaluated in *Orobanche* infested fields and pot experiments in Tigray, Ethiopia. The aim of the study was to determine the critical stage of host plants affected by parasite and to evaluate resistance level of faba bean genotypes. The degree of infection and host resistance level was evaluated at three host growing stages (flowering, pod setting, and maturity stages) using different traits like number of *Orobanche* emerged per plant, per plot, incidence, and severity. The agronomic data such as stand count at emergence, flowering, pod setting, maturity, plant height, pod number, seed per pod, hundred seed weight, and grain yield were recorded from five and three randomly selected plants in the field and pot experiments, respectively. The analysis of variance showed that there were high significant variations ($p < 0.01$) in measured traits between the three host growing stages and between genotypes in agronomic traits. The effect of *O. crenata* on host plant was started from the flowering stage, but the pod setting stage is economically important stage at which actual effect of the parasite was observed both at field and pot experiments. Based on the result of the study, all tested traits at field and pot experiments allowed separating the faba bean genotypes into three groups: partially resistant and or tolerant genotypes "Ashange, Dide'a, and Obse," moderately susceptible genotypes "Holleta, Selale, Wayu, Welki, Mesay, Bulga, Degaga, Gachena, Mosise, and Shalo," and highly susceptible genotypes "Moti, Gebelcho, Dosha, Tumsa, Hachalu, and Tesfa Aloshe."

1. Introduction

Broomrapes (*Orobanche* sp.) are dangerous root parasites for legume crops and only germinate in response to specific stimulant chemicals released by the host plant. Following germination, the seedlings attach to the host roots by the production of specialized feeding structures called haustorium that form a functional bridge to their hosts, which serves as a connection through which water and nutrients are driven from the host to the parasite [1]. Broomrapes devote most of their life cycle underground haustoria to penetrate the host tissues until they reach the vascular system for uptake of water, nutrients assimilate, and grow at the expense of the host plant's resources [2]. A single broomrape plant can release over 500,000 seeds, which are known to remain viable for decades in the soil. This provides the parasite with a great genetic adaptability to environmental changes, including host resistance, agronomical practices, and herbicide treatment [2].

Several scientists from different countries have contributed to investigate the behavior and effect of *Orobanche* spp. as well as the production of new varieties that are significantly less susceptible to the parasite and more productive in highly infested fields. However, the mechanisms involved during plant defense in response to *Orobanche* spp. are complex, and except in the case of chickpea and pea,

some resistance traits were found giving new hopes of genetic improvement in these crops [3–5], total resistance to *Orobanche* is rarely observed [4, 6].

In Ethiopia, there are two broomrape species (*O. crenata* and *O. ramosa*) that attack mainly legume crops and cause the yield losses up to 100% [7]. However, *O. crenata* is broadly distributed and has a wide host range which causes substantial losses and damage in pulse crops, especially faba bean. Recently, it is threatening the livelihood of the farmers, particularly in northern parts of the country with its devastating effect on faba bean which is widely used by farmers [8].

The main dispersal ways of *O. crenata* seeds are through human beings, animals, agricultural tools, and host seeds. The uncertified seed used by farmers, uncontrolled movements of grazing animals, and farm equipment from infested to noninfested fields are the most important spreading agent of this parasite weed [9]. Several management methods like cultural, chemical, and biological have been devised to solve the *Orobanche* problem, but the majority are either too expensive or only partly effective [10]. This is due to the difficulty and nature of the parasite; the infection and pathogenesis processes occur underground, rapid production, and distribution. The only management option preferred to minimize the effect of this parasite is hand weeding other than other control methods. Hence, the appropriate time of weeding should be determined for the hand weeding management to be effective to reduce the infestation of *O. crenata* on faba bean production. Therefore, the aim of the present study was to identify the critical stages of host plants affected by *O. crenata*, thereby to assess the resistance level of faba bean genotypes to the parasite.

2. Materials and Methods

2.1. Experimental Conditions. The experiment was conducted at South Tigray, Ethiopia, both under the naturally infested field and controlled conditions. In field experiment, the study was carried out at Alamata Agricultural Research Center experimental site during the seasons of 2017 and 2018 GC. The experimental field is located at an altitude of 2,600 m.as.l with 12°31′N latitude and 39°33′E longitude having annual rainfall ranging from 600 to 1,000 mm and brown soil type with a pH of 6.5 [8].

In pot experiment, the degree of *O. crenata* infection and its reaction with twenty faba bean varieties was evaluated in greenhouse using pot (17 × 18 cm diam.). Seeds of *O. crenata* were collected from the infected field, where the field experiment was conducted and kept in the dark at 25°C until used for experimental infestations. Seeds of faba bean and *O. crenata* were surface-sterilized by incubation in 1% calcium hypochlorite for 10 min and washed twice with sterilized water before use to make contamination free [11]. For each genotype, three seeds of faba bean were grown in pots containing sterilized soil and river sand (2 : 1) artificially inoculated with 5 mg of parasite seeds per pot [12]. Plants were grown in greenhouse under natural light at 22°C.

2.2. Experimental Materials and Design. Twenty faba bean varieties were used for this investigation. The Ashange variety was used as resistance control [13] which obtained from Alamata Agricultural Research Center. All other varieties were evaluated for their response to *Orobanche* in highly infested soils in field experiments and artificially inoculated in the pot experiments. The experiment was done in three replications of 1.5 m apart using randomized complete block design (RCBD) in field and completely randomized design (CRD) in pot experiments. Each plot consists 4 rows of 3 m length, with 0.1 m and 0.4 m space between plant and row, respectively (4.8 m^2 plot size), in field study. All culture practices such as fertilizer application and hand weeding except for *Orobanche* and hoeing were applied during the growing of crops, but no chemical treatment (herbicide) was applied.

2.3. Data Collection and Analysis. Data collection was carried out on plant basis and plot basis in field experiments. Data for plant height, number of pods per plant, and number of seeds per pod were collected on the basis of five randomly selected plants from two central rows; whereas, stand count at emergence and maturity, days to 50% flowering, days to 90% maturity, grain yield, hundred seed weight, and number of emerged *Orobanche* spike per plant, were collected on the basis of net plot. Ability of each host genotype stand with *O. crenata* was also estimated in the infested field through the incidence of parasitism and a severity index. Incidence was estimated using a 0 to 100% scale; 0% represented a row in which no *O. crenata* had emerged, and 100% represented a row in which all the host plants carried emerged spikes of *O. crenata*. Severity was estimated using a 1 to 9 scale, in which 1 represented healthy plants with no carrying emerged parasite (high resistance) and 9 represented dead host plants with extensive parasite emergence (high susceptibility) [14].

In pot experiment, the number of emerging *Orobanche* spikes per plant (NEO/P) and number of host plants viable were recorded at the three host growing stages (at flowering, pod setting, and maturity stages). Contrary to field experiment, there was no number of subterranean broomrapes considered in pot experiment because the inoculated seeds are emerged only and attached with host plant. All the collected data from both field and pot experiments were subjected to ANOVA by using SAS version 9.2. The significance of the mean difference between varieties was evaluated by the Tukey's test "honestly significant difference test" (HSD) at $p = 0.05$.

3. Results

3.1. Degree of O. crenata Infection on Faba Bean and Host Responses during Growing Stages. The results from the analysis of variance revealed that there were significant ($p < 0.01$) variations among faba bean varieties in their response to *O. crenata* for the most studied parameters (Tables 1 and 2). During the study, all measured traits regarding to *O. crenata* infestation such as number of

Table 1: List of genotypes.

Genotypes name	Pedigree	Origin	Year of release	Breeder/maintainer
Aloshe	—	Ethiopia	2017	Sinana ARC
Ashange	ILB 4358	Morocco	2015	Alamata ARC
Bulga-70	—	Ethiopia	1994/95	Kulumsa ARC
Dide'a	EHO 1048-1	Ethiopia	2014	Kulumsa ARC
Degaga	R-878-3	ICARDA	2022	Holleta ARC
Dosha	COLL 155/00-3	Ethiopia	2009	Holleta ARC
Gachana	ETH91001-13-2	Ethiopia	2008	Haramaya University
Gebalcho	EH 96009-1	Ethiopia	2006	Holleta ARC
Hachalu	EH 0 0 102-4-1	Ethiopia	2010	Holleta ARC
Holleta-2	BP 1802-1-2	ICARDA	2001	Holleta ARC
Mesay	—	Ethiopia	1995/96	Sinana ARC
Mosise	EH-99047-1	Ethiopia	2013	Sinana ARC
Moti	EH 95078-6	Ethiopia	2006	Holleta ARC
Obse	EH95073-1	Ethiopia	2007	Holleta ARC
Salale	Selale Kasim 91-13	Ethiopia	2002	Holleta ARC
Shalo	EH011-22-1	Ethiopia	2000	Sinana ARC
Tesfa	75 TA 2626-1-2-1	ICARDA	1995/96	Holleta ARC
Tumsa	EH99051-3	Ethiopia	2010	Holleta ARC
Wayu	Wayu 89-5	Ethiopia	2022	Holleta ARC
Welki	EH96049-2	Ethiopia	2008	Holleta ARC

ARC=Agricultural Research Center.

Table 2: Mean square of number of *Orobanche* emerged (NOE) at different host growing stages and its incidence percentage and severity in an infested field.

Source of variations	Degree of freedom	*Mean square*								
		NOEpF	NOEpPS	NOEpMt	NOEplotF	NOEplotPS	NOEplotMt	AvgNOEPlot	OI	S
Genotypes	19	0.90**	11.31**	2.82**	3249.4**	13480.23**	739.0**	2361.34**	1046.3**	7.70**
Error	38	0.038	0.67	0.04	96.57	736.0	25.78	103.1	10.75	0.41
R-square		0.92	0.895	0.97	0.94	0.902	0.94	0.92	0.99	0.97
CV (%)		15.8	12.8	13.5	13.33	10.84	29.9	8.9	4.1	8.57
Mean		1.23	6.41	1.51	73.74	250.21	16.98	113.64	79.97	7.48

**Significant at $p < 0.0001$.

Orobanche spike emerged per plant, per plot, incidence, and severity were evaluated by separating the host developmental stage into three, in which high significant variation was observed between the three growing stages.

At the host flowering stage, the effect of *O. crenata* infestation on faba bean genotypes was comparatively lower due to the minimum number of emerged parasites. But, in rare cases, a number of *Orobanche* spikes (less than 2) emerged within single plant of highly susceptible host like Aloshe, Gebelcho, Moti, and Tesfa Tumsa. However, the infection level of the parasite on faba bean was estimated about 10% which is economically not visible at this host growing stage.

However, amongst the three host growing stages, a host pod setting stage is the one at which the maximum infestation of the parasite was observed and the host susceptibility level was clearly identified and a high significant variation in *Orobanche* infestation was observed (Figures 1(a) and 1(b)). Even though, different in number of *Orobanche* emerged per plant from variety to variety, there were no free-parasite attached varieties observed at all. However, a significantly minimum number of emerging parasites was recorded from resistance genotype "Ashange" (Table3). This genotype showed a good adaptation under *Orobanche*-prone areas [7] and demonstrated as good level of resistance to *O. crenata* [15] in Ethiopia.

At the host maturity stage, the number of emerged *Orobanche* spikes per faba bean plant was contrary to at flowering and pod setting stages; that the large number of emerged parasite shoots per plant was observed in the varieties considered as tolerant or/resistant to the parasite like Ashange, Dide'a, and Obse, varieties (Figure 1(c)). The number of parasite shoots per plant recorded from these varieties was ranged 3–4.30 (Table 3), but no significant effect on yield because of the pod already physiologically matured.

The intermediate value of 1-2 *Orobanche* per plant was recorded from the varieties of Bulga-70, Gachena, Salale, Wayu, Mesay, Mosissie, Holetta-2, Degaga, and Welki varieties. However, in highly susceptible varieties, the number of emerging parasites was decreased at maturity stage, due to the host plants already died at this stage (Figure 1(d)). In general, regardless the level of infestation was varied from one growing stage to another the host plant was challenged starting from vegetative growth up to maturity with overall incidence ranging 38.5%–100% and severity score 3.3–9 scale in tested faba bean varieties during the field experiment (Table 3).

(a) (b)

(c) (d)

Figure 1: Level of *O. crenata* infestation per faba bean at different growing stages in infested field; (a) *O. crenata* spike per single plant root of susceptible host at pod setting stage, (b) *O. crenata* spike emerged per total plot in susceptible host at pod setting stage, (c) *O. crenata* spike emerged per plot in tolerant host at maturity stage, and (d) susceptible host at maturity stage.

3.2. Resistance and Susceptibility Level of Host Plant to the Parasite. The resistance and susceptibility level of faba bean varieties was also evaluated by using different agronomic parameters both at field and pot experiments. The analysis of variance from field trial shows significant differences among the genotypes in all tested traits (Tables 2 and 4) that the *Orobanche* infestation was exerted high impact on the tested agronomic variables. Significant differences in infection level and tested agronomic traits among the varieties can be ascribed to variations in the ability of host plant to stand with the parasite. Similar results were also reported by Rubiales et al. [5].

The host stand count at different developmental stages and number of emerged *Orobanche* spike were the key parameters than the other traits in this study, when the other left traits were determined depend on these two parameters, i.e., higher in number of host plant reach for pod maturity and low in number of emerged parasite is higher in yield and yield components. Based on this phenomenon, the higher number of plant at harvested, and high in plant height, pod per plant, seed per pod, hundred seed weight, and grain yield were recorded from the genotypes of Ashange, Dide'a, and Obse followed by Degaga variety (Table 5), while the lower value of the aforementioned traits was recorded from Bulga-70, Gachena, Holleta-2, Mesay, Mosise, Selale, Wayu, and Welki, whereas no grain yield was recorded from the left genotypes. In all cases, however, the variety used as resistance control "Ashange" had better performed than all other genotypes followed by Dide'a and Obse.

Days to flowering and maturity were also the other traits evaluated to test the effect of *Orobanche* infestation on faba bean crop. The result from analyzed data was revealed that

Table 3: Mean number of *Orobanche* emerged spikes per plants and its severity and incidence infestation on faba bean genotypes under an *O. crenata*-infested field.

Genotypes	NOE/PtF	NOE/PtPs	NOE/PtMt	NOE/PlF	NOE/PlPs	NOE/PlMt	Avg.NOE/Pl	OI	OS
Aloshe	1.93^{a}	8^{bc}	0.53^{jk}	124^{a}	316.67^{b}	3.33^{gh}	148^{bc}	98.33^{a}	9^{a}
Ashange	0.00^{g}	1.53^{g}	3.00^{b}	0.00^{i}	71.60^{j}	55.33^{a}	42.31^{i}	36.67^{i}	3.00^{g}
Bulga-70	1.27^{b}	4.67^{f}	2.00^{c}	76.00^{d}	199.00^{hi}	24.00^{cd}	99.67^{gh}	74.67^{def}	7.33^{bcd}
Dide'a	0.67^{f}	5.67^{def}	3.20^{b}	38.33^{h}	182.33^{i}	41.33^{b}	87.33^{h}	50.00^{h}	5.00^{f}
Degaga	1.07^{bcd}	6.40^{de}	1.20^{fgh}	65.67^{def}	253.33^{cdef}	18.00^{cde}	112.33^{efg}	68.70^{g}	7.00^{cd}
Dosha	0.87^{def}	6.40^{de}	0.80^{ijk}	53.73^{fgh}	267.00^{cde}	4.67^{fgh}	108.47^{efg}	100.00^{a}	9.00^{a}
Gachana	1.00^{bcde}	7.00^{cd}	1.80^{cd}	59.00^{efg}	283.67^{bcd}	15.33^{e}	119.33^{def}	77.67^{cde}	7.00^{cd}
Gebalcho	1.93^{a}	6.73^{cd}	0.93^{hi}	116.00^{abc}	249.67^{defg}	4.00^{fgh}	123.22^{de}	100.00^{a}	9.00^{a}
Hachalu	1.80^{a}	7.00^{cd}	0.73^{ijk}	101.00^{c}	296.00^{bc}	4.00^{fgh}	133.67^{cd}	100.00^{a}	9.00^{a}
Holleta-2	1.07^{bcd}	6.67^{cde}	1.33^{efg}	63.33^{def}	244.67^{defg}	12.33^{ef}	106.78^{efg}	76.05^{de}	7.67^{bc}
Mesay	1.2^{bc}	4.67^{f}	1.47^{def}	71.00^{de}	208.33^{ghi}	17.67^{cde}	99.00^{gh}	73.94^{defg}	7.00^{cd}
Mosise	0.93^{cdef}	5.33^{ef}	1.53^{def}	58.33^{efg}	235.67^{efgh}	14.67^{e}	102.89^{fgh}	78.85^{cd}	7.67^{bc}
Moti	1.93^{a}	9.00^{ab}	0.87^{hij}	107.33^{bc}	326.00^{ab}	3.67^{gh}	145.67^{c}	100.00^{a}	9.00^{a}
Obse	0.73^{ef}	4.67^{f}	4.27^{a}	44.33^{gh}	216.00^{fghi}	50.67^{a}	103.67^{fgh}	53.33^{h}	5.67^{ef}
Salale	0.93^{cdef}	5.73^{def}	1.60^{de}	55.33^{efg}	208.33^{ghi}	26.00^{c}	96.56^{gh}	70.06^{fg}	6.33^{de}
Shalo	1.07^{bcd}	6.00^{def}	1.00^{ghi}	67.33^{def}	220.67^{fghi}	9.67^{efgh}	99.22^{gh}	86.12^{b}	8.33^{ab}
Tesfa	2.07^{a}	10.07^{a}	0.47^{k}	127.00^{a}	368.00^{a}	2.67^{h}	165.89^{a}	100.00^{a}	9.00^{a}
Tumsa	2.00^{a}	10.00^{a}	0.93^{hi}	122.00^{ab}	363.67^{a}	3.67^{gh}	163.11^{ab}	100.00^{a}	9.00^{a}
Wayu	1.07^{bcd}	6.33^{de}	1.47^{def}	63.00^{def}	244.33^{defg}	17.33^{de}	108.22^{efg}	73.15^{efg}	7.00^{cd}
Welki	1.07^{bcd}	6.27^{de}	1.00^{ghi}	62.00^{def}	249.33^{defg}	11.33^{efg}	107.56^{efg}	81.88^{bc}	7.67^{bc}
LSD (5%)	0.32	1.35	0.34	16.24	44.84	8.392	16.78	5.42	1.06

Means with the same letter are not significant difference. NOE/PtF = number of *Orobanche* emerged per plant at host flowering stage, NOE/PtPs = number of *Orobanche* emerged per plant at host pod-setting stage, NOE/PtMt = number of *Orobanche* emerged per plant at host maturity stage, NOE/PlF = number of *Orobanche* emerged per plot at host flowering stage, NOE/PlPs = number of *Orobanche* emerged per plot at host pod-setting stage, NOE/PlMt = number of *Orobanche* emerged per plot at host maturity stage, and AvgNOE/Pl = average number of *Orobanche* emerged per plot, *Orobanche* incidence, *Orobanche* severity.

Table 4: Analysis of variation (ANOVA) for yield and yield attributed traits of faba bean varieties evaluated under *O. crenata*-infested field.

Source of variations	Degree of freedom	*Mean square*										
		SCE	SCF	SCPs	SCMt	DF	DM	PH	NPP	NSP	HSW	GY t/ha
Genotypes	19	28.5^{**}	24.6^{**}	27.8^{**}	184.7^{**}	9.3^{**}	9271.5^{**}	283.4^{**}	61.3^{**}	7.3^{**}	2729.0^{**}	0.454^{**}
Error	38	3.42	4.68	1.91	0.8	0.61	1.51	28.86	0.34	0.006	1.57	0.0002
CV (%)		2.6	3.61	3.42	10.16	1.57	1.49	8.03	10.2	3.99	3.4	4.36
R-square		0.81	0.72	0.88	0.99	0.88	0.99	0.83	0.99	0.99	0.99	0.99

**Significant at $p < 0.001$. SCE = stand count at emergence, SCF = stand count at flowering, SCMt = stand count at maturity, PH = plant height, NPP = number of pods per plant, NSP = number of seeds per pod, HSW = hundred seed weight, and GY/t/ha = grain yield in ton per hectare.

there were significant variations in days to flowering and maturity between the genotypes, but there is no more effect of *Orobanche* for the variation in days to flowering rather than due to genetic makeup of the genotypes. In some cases, some varieties Gachena, Mesay, Selale, and Wayu, which considered as susceptible genotypes were earlier in days to maturity than the other varieties with days of 113.3, 115, 115.7, and 115.7, respectively, that the others varieties which took 117.7–122 days from planting (Table 5). This might enable these genotypes to scape due to their short growth cycle. Meanwhile, it was difficult to measure days to maturity for highly susceptible genotypes, because they were totally destroyed before reaching pod maturity.

The pot experiment through application of an artificial inoculation of the parasite seeds to host plant in pots under a greenhouse was carried out to confirm or reject the result obtained from field experiment. During this experiment, the parasite-host reaction was evaluated at three host growing stage with similar procedure of field experiment. Accordingly, at the first part of the growing stage (Flowering stage) the establishment of *O. crenata* was rarely observed on genotypes, whereas no parasite spikes showed on genotypes Ashange and Dide'a at all. Later on, showing *O. crenata* formation on all genotypes at pod setting stage with different degree of infection, and continued to observe on host plant at maturity stage which is a disadvantage for the parasite (Table 6). The number of host plants counted at emergence and at maturity was also the most important way to determine the susceptibility level of the varieties to the parasite in pot experiments; this is because the highly susceptible genotypes were not reaching for maturity and significant variations were observed among the genotypes in some traits (Table 6).

3.3. Simple Linear Regression Analysis for Faba Bean Yield with Mean Orobanche Emerged per Plot during the Three Host Growing Stages (Flowering, Pod Setting, and Maturity Stages). The regression analysis in this study focused on yield prediction for each faba bean genotypes conducted in

Table 5: Mean performance of yield and yield attributed traits of faba bean genotypes in an *O. crenata*-infested field.

Genotypes	SCE	SCF	SCPs	SCMt	DF	DM	PH	NPP	NSP	HSW	GY
Aloshe	72^{abcde}	64.0^{ab}	39.3^{fgh}	0.0^{i}	48.7^{fgh}	0.0^{i}	58.4^{ghi}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Ashange	69.67^{defg}	65.3^{a}	48.3^{a}	26.3^{a}	50.3^{cd}	122.0^{a}	90.03^{a}	11.7^{a}	4.0^{a}	65.0^{d}	1.56^{a}
Bulga-70	67.67^{fghij}	60.7^{bcde}	43.0^{bc}	12.0^{e}	49.3^{def}	119.3^{bcde}	64.8^{efg}	8.0^{d}	2.0^{d}	50.0^{f}	0.19^{i}
Dide'a	66.33^{hij}	54.7^{i}	41.0^{cdef}	19.7^{b}	49.0^{efg}	119.3^{bcde}	80.3^{b}	11.0^{ab}	3.0^{c}	68.3^{c}	0.85^{b}
Degaga	72.67^{abcd}	61.7^{bcd}	39.0^{fghi}	15.3^{d}	50.0^{cde}	118.7^{de}	78.0^{bc}	10.3^{b}	3.0^{c}	59.7^{e}	0.58^{d}
Dosha	73.33^{abc}	62.0^{abcd}	41.7^{cde}	0.0^{i}	52.7^{a}	120.7^{abcd}	65.0^{efg}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Gachana	68.33^{fghij}	59.0^{defgh}	40.0^{efg}	8.7^{h}	47.3^{i}	113.3^{h}	79.5^{b}	7.0^{e}	4.0^{a}	83.7^{a}	0.35^{f}
Gebalcho	73.67^{ab}	60.0^{cdef}	37.0^{ij}	0.0^{i}	47.7^{hi}	0.0^{i}	51.97^{i}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Hachalu	65.33^{j}	56.0^{ghi}	42.3^{cd}	0.0^{i}	48.0^{ghi}	0.0^{i}	58.6^{ghi}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Holleta-2	68.67^{fghi}	59.3^{defg}	37.7^{hij}	$8.\ 7^{h}$	49.7^{def}	119.0^{cde}	67.5^{def}	11.0^{ab}	3.0^{c}	38.0^{h}	0.19^{i}
Mesay	69.0^{efgh}	59.33^{defg}	45.0^{b}	12.0^{e}	51.7^{ab}	115.0^{gh}	62.2^{efgh}	8.3^{d}	3.0^{c}	46.0^{g}	0.28^{g}
Mosise	70.33^{cdefg}	63.0^{abc}	43.0^{bc}	9.7gh	50.0^{cde}	121.0^{abc}	74.3^{bcd}	7.0^{e}	3.0^{c}	60.0^{e}	0.24^{h}
Moti	66.00^{hij}	55.7^{hi}	36.3^{j}	0.0^{i}	47.0^{i}	0.0^{i}	69.0^{def}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Obse	74.67^{a}	57.0^{fghi}	43.0^{bc}	$16.\ 7^{cd}$	48.0^{ghi}	117.7^{ef}	70.4^{cde}	7.7^{de}	3.8^{b}	77.0^{b}	0.71^{c}
Salale	65.67^{ij}	59.0^{defgh}	39.0^{fghi}	17.0^{c}	49.0^{efg}	115.7^{fg}	67.6^{def}	8.3^{d}	3.0^{c}	48.7^{f}	0.43^{e}
Shalo	73.00^{abc}	63.3^{abc}	38.0^{ghij}	8.3^{h}	52.0^{ab}	117.7^{ef}	62.3^{efgh}	7.7^{de}	2.0^{d}	60.7^{e}	0.16^{j}
Tesfa	70.67^{bcdef}	61.7^{bcd}	37.0^{ij}	0.0^{i}	48.0^{ghi}	0.0^{i}	55.5^{hi}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Tumsa	75.00^{a}	61.0^{bcde}	38.0^{ghij}	0.0^{i}	51.0^{bc}	0.0^{i}	60.3^{fghi}	0.0^{f}	0.0^{e}	0.0^{i}	0.0^{k}
Wayu	70.67^{bcdef}	59.0^{defgh}	39.0^{fghi}	11.3^{ef}	52.0^{ab}	115.3^{gh}	55.3^{hi}	9.3^{c}	3.0^{c}	40.0^{h}	0.25^{h}
Welki	67.33^{ghij}	58.0^{efghi}	40.3^{def}	10.3^{fg}	52.0^{ab}	121.3^{ab}	66.3^{defg}	7.7^{de}	2.0^{d}	49.7^{f}	0.16^{j}
LSD	3.06	3.58	2.28	1.48	1.29	2.03	8.88	0.97	0.13	2.07	0.022

Means with the same letter are not significant difference. SCE = stand count at emergence, SCF = stand count at flowering, SCMt = stand count at maturity, PH = plant height, NPP = number of pods per plant, NSP = number of seeds per pod, HSW = hundred seed weight, and GY/t/ha = grain yield in ton per hectare.

Orobanche infested field by using simple linear regression model that the prediction equation was formulated as $Y = \alpha + \beta X + e$ [16].

(i) Y is the predicted value of the dependent variable (y) for any given value of the independent variable (x).

(ii) α is the intercept, the predicted value of y when the x is 0.

(iii) β is the regression coefficient, how much the expected y to be changed as x increases.

(iv) X is the independent variable (the variable we expect is influencing y).

(v) e is the error of the estimate, or how much variation there is in our estimate of the regression coefficient.

In this case, the grain yield was a dependent/response variable, while the mean number of *Orobanche* emerged per plot from the three host-growing stages used as independent/explanatory variable. The predicted value for grain yield in this analysis was calculated as "$Y = -0.0108x + 1.53$," when −0.0108 is the regression coefficient or slop (m), 1.53 is intercept (b), y and x are represented as predicted grain yield and the given (observed) value of *Orobanche* emerged per plot, respectively.

The analysis of variance from regression analysis (Table 7), therefore, revealed that strong linear relationship (78.45%) between predicted and predictor variables expressed as multiple R (Pearson correlation coefficient value) and high significance variation among faba bean genotypes ($p < 0.01$), successfully accounted for 61.5% of the total variation of grain yield was by the *Orobanche* infestation expressed as R^2. The residual content (38.5%) may be attributed to other studied agronomic traits, unknown variation (random errors) and human errors during measuring the studied traits and/or some other traits that were not included in this study. This implies that the response variable was highly influenced by the explanatory variable.

Graphical analysis of the data showed negative slop and positive intercept of the regression line which the linear regression coefficient was estimated −0.011. Distribution of the scatter points in the graph represented the measured grain yield of each faba bean genotype and their genetic constitution to resist the parasite infestation, while distribution of the numbers in the graph represented the tested genotypes (observation number). The linear regression line represented the predicted grain yield. Linear regression finds the line of best fit line through the dependent/response and independent/explanatory variables data by searching for the regression coefficient (β) that minimizes the total error (e) of the model. All genotypes which located below the regression line were had negative residuals when all with positive residuals were located above the regression line (Figure2). Negative residuals imply greater predicted values than measured values, and it is calculated by "Actual value-predicted value."

4. Discussion

The aim of the study was to determine the critical stage of host plants affected by *O. crenata* by examining the degree of the parasite infection on faba bean varieties at three host

Table 6: Total number of host seeds planted (NHSP), total number of *Orobanche* per plant (TNO/P), and number of viable host plants per pot (NVH/P) at three host-growing stages in the pot experiment.

		At flowering		*At pod setting*		*At maturity*	
Genotypes	NHSP	TNOE/P	NVH/Po	TNOE/P	NVH/Po	TNOE/P	NVH/Po
Aloshe	3.00	2.11^{a}	3.00	3.83^{bc}	2.67^{ab}	0.00^{d}	0.00^{f}
Ashange	3.00	0.00^{h}	3.00	0.89^{j}	3.00^{a}	2.33^{bc}	3.00^{a}
Bulga	3.00	0.22^{gh}	3.00	2.78^{h}	3.00^{a}	2.33^{bc}	0.67^{ef}
Dide'a	3.00	0.00^{h}	3.00	1.56^{i}	3.00^{a}	3.78^{ab}	2.67^{ab}
Degaga	3.00	0.44^{fg}	3.00	3.33^{cdefg}	3.00^{a}	4.33^{a}	1.67^{cd}
Dosha	3.00	1.00^{cd}	3.00	3.56^{cde}	3.00^{a}	0.00^{d}	0.00^{f}
Gachana	3.00	0.78^{de}	3.00	2.89^{gh}	3.00^{a}	2.17^{bc}	1.00^{de}
Gebalcho	3.00	1.44^{b}	3.00	3.11^{efgh}	3.00^{a}	0.00^{d}	0.00^{f}
Hachalu	3.00	1.44^{b}	3.00	3.44^{cdef}	3.00^{a}	0.00^{d}	0.00^{f}
Holleta	3.00	0.78^{de}	3.00	3.44^{cdef}	3.00^{a}	4.33^{a}	1.67^{cd}
Mesay	3.00	0.67^{ef}	3.00	3fgh	3.00^{a}	3.83^{ab}	1.67^{cd}
Mosise	3.00	1.22^{bc}	3.00	3.11^{efgh}	3.00^{a}	3.5^{ab}	1.33^{cde}
Moti	3.00	2.22^{a}	3.00	4.39^{a}	2.33^{b}	0.00^{d}	0.00^{f}
Obse	3.00	0.11^{h}	3.00	2.00^{i}	3.00^{a}	4.44^{a}	2.67^{ab}
Salale	3.00	0.44^{fg}	3.00	$3.22d^{efgh}$	3.00^{a}	2.94^{abc}	2.00^{bc}
Shalo	3.00	0.78^{de}	3.00	3.56^{cde}	3.00^{a}	1.5^{cd}	0.67^{ef}
Tesfa	3.00	2.22^{a}	3.00	4.17^{ab}	2.67^{ab}	0.00^{d}	0.00^{f}
Tumsa	3.00	1.00^{cd}	3.00	3.67^{bcd}	3.00^{a}	2.67^{abc}	0.67^{ef}
Wayu	3.00	0.22^{gh}	3.00	3.44^{cdef}	3.00^{a}	4.00^{ab}	1.33^{cde}
Welki	3.00	0.55^{ef}	3.00	3.33^{cdefg}	2.67^{ab}	4.33^{a}	1.00^{de}
LSD	0.00	0.33	0.00	0.53	0.401	1.89	0.94
Pr	NS	*	NS	**	NS	**	**

Means with the same letter are not significant difference.

Table 7: ANOVA of simple linear regression analysis.

Sources	d*f*	Regression coefficient (m)	MS	Standard error	*t*-value	*p* value	Lower 95%	Upper 95%
Regression	1	−0.0108	1.76^{**}	0.002	28.81	0.000042	−0.015	−0.007
Residual	18		0.061					
Intercept (*b*)				1.53				
Multiple *R*				0.785				
R^2				0.6155				
Adjusted R^2				0.59412				

growing stages thereby to develop resistant variety to the parasite. In previous investigations regarding the effect of *Orobanche* infestation on faba bean, different authors used several criteria to quantify resistance or tolerance to the parasite. According to the author in [17], the number of *Orobanche* per host plant, height of parasitic shoots, and number of *Orobanche* per sown surface unit were the major criteria, whereas some authors [5, 18] suggested that the number of *Orobanche* shoots per host plant is the best index, which gives the most reliable estimation of the total level of infestation. However, other authors [19] suggested that a screening based only on the number of emerged shoots was misleading, and other traits like the health of the host plants must also be considered to effectively use the management method to be applied.

In this study, therefore, in addition to the number of *Orobanche* shoots emerged per host plant, the incidence and severity of parasite, different host growing stages, and host stand count were the main parameters have been used. The level of *O. crenata* infestation was determined by categorizing the host plants into three growing stages (at flowering, pod setting, and maturity stages) using different parameters on *Orobanche* spikes and on host plants. Contrary to this, Perez-de-Luque et al. [1] investigated for *O. crenata* resistance in pea by operating different developmental stages of the parasite, which results with different *Pisum* genotypes showed that resistance is the result of several mechanisms acting at different stages of the parasite infection process. In the present study, based on the result obtained from the mean of parasite infestation at each growth stage the reliable critical stage at which host plants highly affected was clearly identified and the susceptibility level of the host plants was also determined. Significant variations in the measured traits between the three growing stages can be attributed to variations in the stage of host plants critically influenced by the parasite. As a result, the degree of *O. crenata* infection on faba bean plant was very high (estimated to about 75%) at pod setting stage whereas about 10% and 15% were at flowering and at maturity stages, respectively.

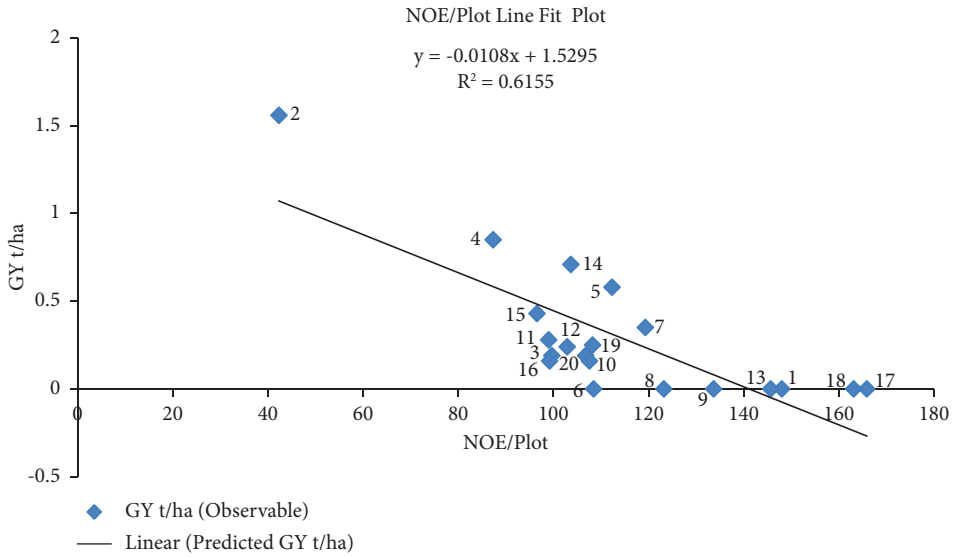

Figure 2: Linear regression model relating number of *Orobanche* emerged per host growing plot against grain yield under *O. crenata*-infested field.

To confirm the comparative stage at which the host was critically criticized and the susceptibility level of the tested genotypes to the parasite, an artificial inoculation experiment was carried out in pots in a greenhouse. The results from this measurement therefore approve the resistant and or tolerance faba bean varieties to *O. crenata* infestation in field experiments. In addition to this, the analysis of variance from regression analysis showed strong linear relationship between the predictor variables of *O. crenata* infestation and the predicted variable of grain yield of faba bean varieties, which implies that the response variable (grain yield) was highly influenced by the explanatory variable (parasite infestation).

Generally, there were the interesting responses in most traits of some varieties (Ashange, Dide'a, and Obse), which are most important varieties that are moderately resistant/tolerant to the parasite, but no complete resistant variety can be observed. Their lower susceptibility was more probably due to a decrease in the number of *Orobanche* attachments and a reduction in parasite emergence, and might be due to their genetic resistance preventing *O. crenata* tubercle formation and development. Similar result was reported by Abbes et al. [11] and Rubiales et al. [4] in pea cultivars. Thus, the results from all parameters taken under evaluation allowed separating the faba bean genotypes into three groups: (a) partially resistant/tolerant varieties "Ashange, Dide'a, and Obse" having comparatively higher grain yield ranged (0.71–1.56 t/ha) with slightly lower *Orobanche* incidence and severity, (b) susceptible genotypes "Bulga, Degaga, Gachena, Holleta, Mesay, Mosise Selale, Shalo, Wayu, and Welki" with lower yield, and (c) highly susceptible genotypes "Aloshe, Dosha, Gebelcho, Hachalu, Moti, Tesfa, and Tumsa" with total (100%) yield loss and >98% and 9 scale of Orobanche incidence and severity, respectively (Tables 3 and 5).

5. Conclusion

In Ethiopia, currently pulse production, especially faba bean is becoming worse due to the wide spread and high infestation of *O. crenata*, causing yield reduction up to 100%, particularly in northern parts of the country. The control of this parasite is so difficult due to its ability to form a bank of seeds in the soil for several years. In the present investigation, several parameters were taken under evaluation by categorizing the host plant in to three growing stages. According to the result obtained from this study, host pod setting stage is economically important stage at which the host critically affected by the parasite. The analyzed data for tested traits, therefore, confirm to categorize the evaluated faba bean genotypes in three different clusters, such as moderately resistance/tolerance, susceptible, and highly susceptible genotypes based on their ability to stand with the parasite. However, of the different traits considered in this study, the host stands count at different developmental stages and number of emerged *Orobanche* spike were the key parameters than the other traits because the other traits were determined based on these two groups of parameters. Accordingly, the varieties Ashange, Dide'a, and Obse were selected as the most important varieties that are moderately resistant and or tolerant to *O. crenata* with higher yield.

Moreover, because of no effective control of the parasite, the most common applying control method in Ethiopia is the hand weeding; there is only a narrow margin between avoiding the parasite alone and damaging the host as well. Thus, further breeding for resistance/or tolerance is considered the best option of control against *Orobanche*. Therefore, it is very important to use the genotypes Ashange, Dide'a, and Obse as parental lines for crossing purpose or intercrossing (crossing each other), until the other accessions will be investigated for better resistance. The other option is focusing on control management or eradication

program that must aim at reducing the seed bank and avoiding the spreading parasite seeds into neighboring fields.

Data Availability

The data that support the findings of this study are openly available in Lemma at https://www.researchgate.net/profile/Lemma-Diriba.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the submission and publication of this article.

Acknowledgments

The author would like to express his deepest thanks to the Ethiopian Institute of Agricultural Research (EIAR) for funding the study, Holleta Agricultural Research Center for providing experimental materials, Mehoni Agricultural Research Center for facilitating to use a budget and other materials, Alemata Agricultural Research Center for support in the experimental sites, and Tigray regional state of agricultural and rural development office for helping in laboratory equipment and endless support to testing the genotypes in laboratory. The Ethiopian Institute of Agricultural Research funded the study, but not for manuscript preparation and publication.

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