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Response of Faba Bean (*Vicia faba*) Genotype to Crenate Broomrape (*Orobanche crenata*) Parasitism

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Abstract

Crenate broomrape (Orobanche crenata Fork.) remains a major biotic-constraint to faba bean (Vicia faba L.) production in northern Ethiopia where small-holder farmers experience substantial yield losses due to the parasitic weed. Despite several research efforts, an effective and practical management recommendation has not yet developed for these farming communities. In response to this challenge, a study was conducted in 2020 at Ambo Agricultural Research Center with the objective of identifying the best performing resistant and locally adapted faba bean genotype that can be used as a component in an integrated crenate broomrape management strategy. A total of 50 faba bean genotype along with known susceptible (Degaga) and partially resistant (Hashenge) check varieties were evaluated under controlled glasshouse condition in the parasite infested soil. The experiment followed a RCBD with three replications. Highly significant variation ($p < 0.01$) was observed among the genotype for all measured parasite infection parameters, including number of emerged shoots, underground tubercles, infection severity and total biomass weight. Among all tested materials, variety 'Hashenge' demonstrated consistent resistance, showing markedly reduced crenate broomrape emergence and biomass. In addition, two elite breeding lines EH96049-10 and EH91016-5-1-1 were showed relatively stable growth and lower susceptibility, indicating their performances were less affected by the parasitism. Therefore, the study confirmed that 'Hashenge' is a valuable variety for inclusion in integrated management targeting crenate broomrape. Furthermore, the elite lines EH96049-10 and EH91016-5-1-1 signify promising source for resistance that can be incorporated into future breeding program of developing new crenate broomrape resistant faba bean variety for affected areas.

Keywords: Host crop, infection, interaction, parasitic weed, reaction

Introduction

Faba bean (*Vicia faba* L.) is the most important cool-season pulse crop grown within Ethiopia's cereal-based production system providing considerable socioeconomic and agronomic benefits to smallholder farmers. The crop accounts for more than 30% of the country's 1.7 million hectares of pulse crop producing land (ESS, 2022). Its seed complements cereal crops diet as a source of protein and minerals for human, and its straw is a good source of animal feed (Meng *et al*., 2021). Fab bean also serves as an important cash crop for farmers and contributes to national foreign-exchange earnings. Agronomically, the crop improves soil fertility through biological nitrogen fixation that assists sustainable crop production when rotated with cereals.

Despite its importance, faba bean production in the highlands of northern part of the country is severely constrained by the obligate root holo-parasitic weed crenate broomrape (*Orobanche crenata* Fork.), which poses a major threat to the livelihood of farmers (Gashaw Belay *et al*., 2020; Takele Negewo *et al*., 2020; Lemma Diriba, 2023). Crenate broomrape is highly invasive, difficult to control, and capable of causing devastating losses to cool-season food and forage legumes. Although it infects a wide range of legume species, the severity of infection varies among species and even among accessions within a species (Fernandez-Aparicio *et al*., 2016). Yield losses can reach 100%, depending on infestation level, host resistance, and environmental conditions (Teklay Abebe *et al*., 2013; Fernandez-Aparicio *et al*., 2016).

Current crenate broomrape management relay mainly on cultural methods and to some extent sub-lethal applications of glyphosate (Mekonnen Misganaw, 2016). Host plant resistance is considered as the most desirable management strategy, however, resistance in faba bean is generally partial with no reports of complete immunity (Rubiales *et al*., 2016; Ennami *et al*., 2017)). In Ethiopia, the first partially resistant faba bean variety ‘Hashenge’ (ILB4358) was released for production in 2015 (Teklay Abebe *et al*., 2015) after evaluation of ICARDA elite lines under heavily crenate broomrape infested conditions.

Identifying high yielding, locally adapted genotype with improved resistance remains essential for sustainable management of the parasite. Therefore, the present study was conducted to evaluate and identify local faba bean genotype capable of resisting crenate broomrape infection under glasshouse condition.

Materials and Methods

Treatments and experimental design

Mature seeds of crenate broomrape were collected from heavily infested faba bean fields located in Korem (south Tigray) and Kutaber (south Wollo). Seeds from the two populations were thoroughly mixed and stored in darkness at room temperature until their biological dormancy period had elapsed, then after used to inoculate the growing media. A total of fifty faba bean genotype along with two standard check varieties; the partially resistant variety ‘Hashenge’ (ILB4358) and the susceptible variety ‘Degaga’ were evaluated for their reaction to crenate broomrape parasitism under glasshouse condition at Ambo Agricultural Research

Center (AmARC) from June to August in 2020. Seeds of the variety Hashenge was sourced from Alamata Agricultural Research Center, whereas seeds of other genotype were obtained from the National Highland Pulse Research Program, Holeta Agricultural Research Center. The pot experiment was arranged in randomized complete block design with three replications in the glasshouse.

Experimental procedures

A soil mixture composed of surface soil, forest soil and sand soil in a 2:1:1 volume ratio was steam-sterilized for 24 hours in an autoclave set at 160 °C temperature. Five-liter plastic pots were each filled with 4 kg of the sterilized soil and then infested with 1.7 g of crenate broomrape seeds into the top 5 cm layer following the procedure of Ennami *et al.* (2017). Five seeds of each faba bean genotype were sown per pot. The pots were irrigated at intervals of two- to three-days, and aluminum coated thermometer was inserted into soil to monitor temperature. After one month, the faba bean were thinned to three seedlings per pot (Figure 1).

Figure 1. Faba bean genotype grown in crenate broomrape infested soil of the glasshouse at AmARC, 2020

Data collection and analysis

Observations of crenate broomrape on faba bean plant were carried out from flowering stage through physiological maturity of the host crop. At physiological maturity of faba bean plants, roots were removed from the pots and washed thoroughly with water. Data were then collected on the crenate broomrape emergence, tubercles formation and parasite biomass, as well as on the lateral root development and biomass of the host crop (Figure 2).


Figure 2. Faba bean infected with crenate broomrape in the glasshouse, AmARC

The infection severity index of the crenate broomrape parasitism was determined based on number of emerged shoots per pot. The average number of emerged crenate broomrape shoots per host crop plant was calculated to assess the severity of infection of the parasite. Crenate broomrape infection severity on each faba bean genotype was classified using a five-point scale adopted from Abbes *et al*. (2011): 0 =no infection (highly resistant), 0.1-0.9 =low emergence (moderately resistant), 1-1.9 =moderate emergence (moderately susceptible), 2.0-3.9 =high emergence (highly susceptible) and $\geq$4 =extensive emergence accompanied by death of host plant (extremely susceptible). Crenate broomrape plant biomass determined by drying shoots and tubercles in an autoclave set at 80 $^{\circ}$C temperature for 48 hours, and recording the dry weight samples. Similarly, the average length of lateral roots per faba bean plant was calculated from root measurements taken for each pot. The dry biomass of the faba bean plants was obtained after drying fresh samples at 80 $^{\circ}$C for 48 hours.

All collected data were analysed using SAS computer software version 9.4 (SAS, 2020). Means comparison were performed using list significant difference (LSD) procedure at the 5% significance level, and correlation analysis was performed among the measured parameters.

Results and Discussion

Test plants physiology: Faba bean plants emerged 10 days after sowing (DAS) and reached the flowering stage between 50 to 60 DAS. Crenate broomrape emergence occurred 40 to 50 days after the faba bean seedlings emerged. As a result, the onset of faba bean flowering and crenate broomrape shoots emergence dates coincide, both occurring around 50 to 60 DAS.

Soil temperature in the pots was recorded at 8:00 am, 12:00 am and 4:00 pm of each day throughout the evaluation period in June, July and August (Table 1). During the experiment. mean maximum and minimum temperatures of 40.50 and 20.19 °C were observed with an overall average temperature value of 26.33 °C. Environmental conditions, particularly temperature has a crucial influence on orobanche development including seed germination. Higher temperatures generally promote orobanche parasitism, while lower temperatures are associated with reduced infections (Soliman *et al*., 2024). The optimal temperature rage for broomrape seed germination is 15 to 25 °C, and germination declines sharply below 5 °C. Therefore, the average soil temperature recorded in the pots was suitable for supporting effective germination and development of the parasite in the presence of the host crop.

Table 1. Potted-soil mean temperature (˚C) recorded at different times of a day during the evaluation months in the glasshouse at AmARC, 2020

Time	Month			Average
	June	July	August	
8 AM	20.20	20.38	20.19	20.26
12 AM	23.50	23.86	25.14	24.17
4 PM	40.50	31.81	31.40	34.57
Mean	28.07	25.35	25.58	26.33

AM=anti-meridian, PM= post-merdiem

Crenate broomrape parasitism parameters on faba bean: Analysis of variance (ANOVA) showed highly significant ($p<0.01$) differences among faba bean genotype for mean number of crenate broomrape shoots, number of tubercles, infection severity and biomass weight (Table 2).

Table 2. Analysis of variance for different parameters of crenate broomrape parasitism on faba bean grown under glasshouse condition

Source	DF*	Mean square			
		# of shoots	# of tubercles	Infection severity	Biomass weight (g)
Block	2	2.43	8.48	0.22	6.43
Genotype	51	21.46**	40.71**	2.44**	53.09**
MS Error	102	1.17	0.87	0.14	1.09
CV		25.66	16.46	26.54	2.79

* DF= degree of freedom, **= highly significant

Number of crenate broomrape shoots: Mean number of emerged crenate broomrape shoots varied widely among the faba bean genotype, ranging from 0 to 13 per pot (Figure 3, Table 3). The lowest mean number of crenate broomrape shoots (<2.00 per pot) were recorded on genotype Salale, Bulga-70, EH91016-5-

1-1, HBP-C-21, HBP-B-10, BLB4342-1, EH00005-3, HBP-E-3, Hashenge and EH96049-10. The variation observed among the faba bean genotype in permitting crenate broomrape seed germination may be attributed to inherent genetic potential. This interpretation aligns with the findings of Rubiales *et al*. (2016), who reported that reduced induction of the parasite seed germination is a key mechanism underlying host resistance to the parasite.

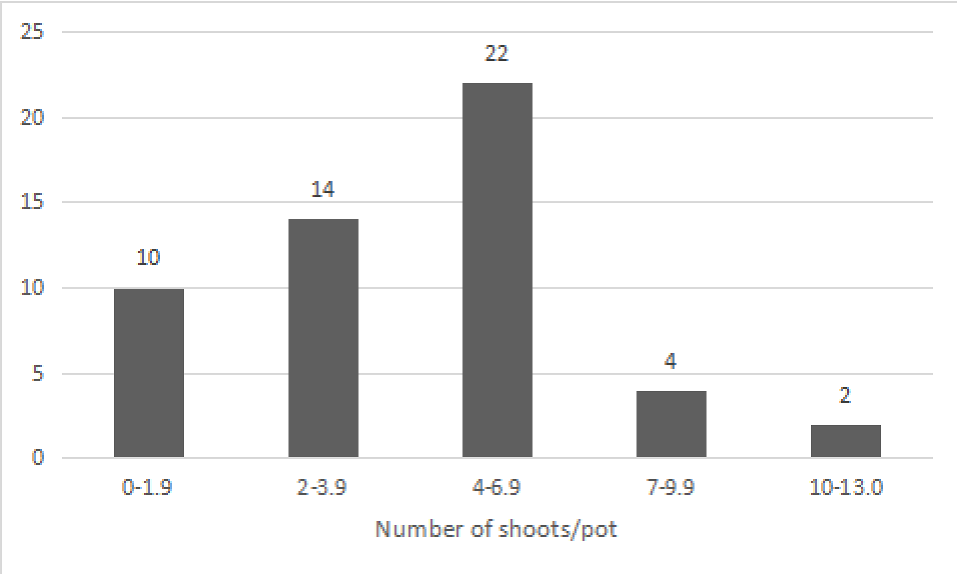

Figure 3. Frequency of faba bean genotype across the different categories of crenate broomrape shoots per pot

Number of tubercles*:* Mean number of crenate broomrape tubercles per pot ranged from 1 to 14.5 (Figure 4, Table 3). The lowest count, ranging from 1 to 1.3 tubercle per pot were recorded from the genotype EH96049-10, EH99001-4, Coll168-00-1, Mesay, EH02216-4, Hashenge, EH91016-5-1-1 and EH43008-Ov3-3. A reduced number of crenate broomrape tubercles attached to faba bean roots reflects a lower infection potential of the parasite or a strong resistance response from the host plant (Trabelsi *et al*., 2016). Potential resistance mechanisms may include cell-wall strengthening, the synthesis of toxic metabolites, or the blockage of vascular connection that limit parasite establishment (Perez-de-Luque *et al*., 2006; Perezde-Luque *et al*., 2007; Lozano *et al*., 2007).

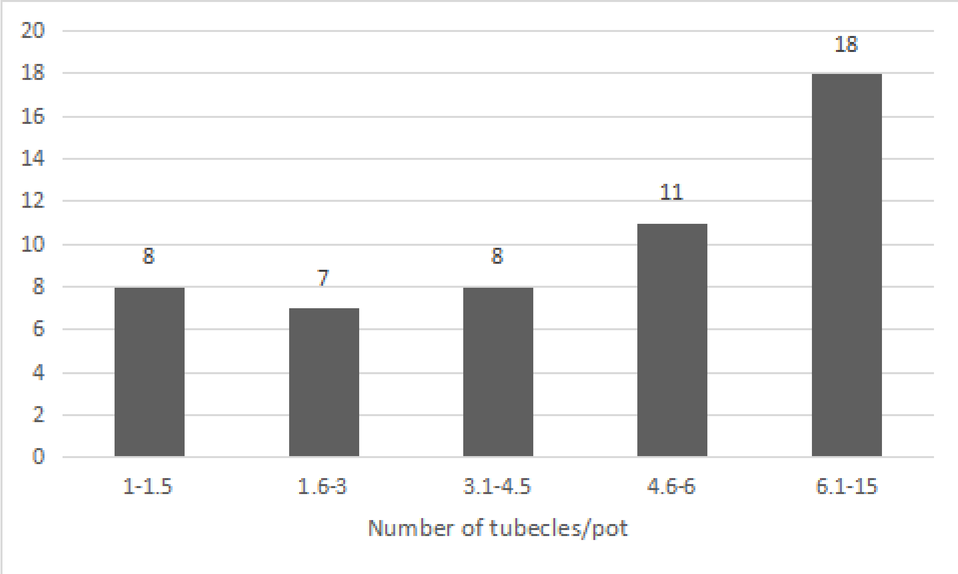

Figure 4. Frequency of faba bean genotype across the different categories of crenate broomrape tubercles per pot

Infection severity*:* Mean severity of crenate broomrape infection on faba bean genotype ranged from 0 to 4.3 (Figure 5, Table 3). Genotype such as Salale, Bulga-70, EH91016-5-1-1, HBP-C-21, HBP-B-10, BLB4342-1, EH96049-10, Hashenge, EH00005-3 and HBP-E-3 exhibited significantly ($p<0.01$) lower infection severity (<0.7) indicating moderate resistance to the crenate broomrape parasitism. Since crenate broomrape is root-parasitic weed, most of its pathogenic activities take place underground; complicating accurate assessment of the levels (Rubiales *et al*., 2016). Lemma Diriba (2023) observed higher level of broomrape infection during host's-pod setting stage, supporting the practice of evaluating infection after pod formation. The significant reduction of crenate broomrape observed in the faba bean genotype such as 'Hashenge', EH96049-10 and EH91016-5-1-1 suggests the presence of promising defence-related genes against this parasitic weed, as explained by Fernandez-Aparicio *et al*. (2016).

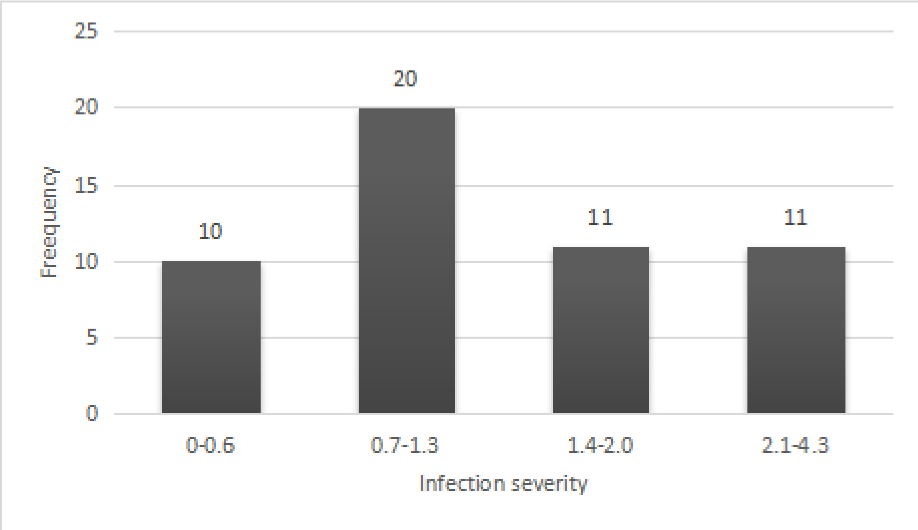

Figure 5. Frequency of faba bean genotype across the different categories of crenate broomrape infection severity

Biomass weight: Mean biomass of uprooted crenate broomrape plants that parasitizing faba bean genotype ranged from 0.20 to 22.83 gramme per pot (Figure 6, Table 3). The lowest biomass weights of below 1 g/pot were observed on genotype EH96049-10, EH02216-4 and EH91016-5-1-1, indicating higher relative resistance of them to the parasitism. Crenate broomrape infection can reduce faba bean plant biomass affecting both growth and more importantly reproductive organs, unless the host exhibits tolerance to the parasite (Amare Birhanu and Birhane Tsehaye, 2020). Resistance is often associated with shorter lateral roots, which limits exposure to the root parasite seed and seedling in the rihizosphere. Furthermore, Tantawy *et al*. (2022) reported that the root length of faba bean genotype was highly significantly and negatively impacted by the number and dry weight of broomrape. This holds true for few genotype such as ‘Mesay’ and ‘Bulga-70’ in the current evaluation of host-parasite interactions.

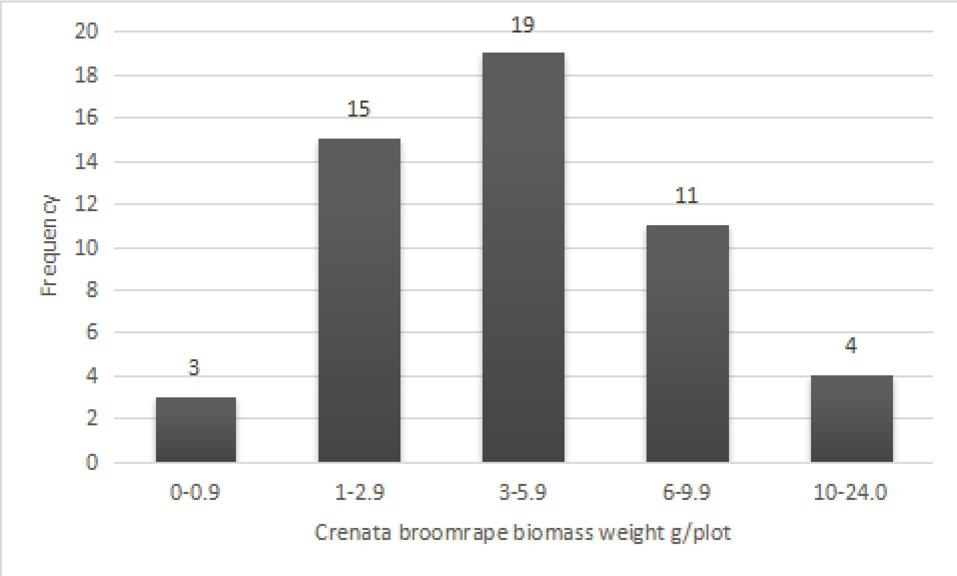

Figure 6. Frequency of faba bean genotype across the different categories of crenate broomrape biomass weight

Table 3. Mean shoot, tubercle, infection and biomass of crenate broomrape as affected by faba bean genotype in the glasshouse at AmARC, 2020

Genotype	# of shoot/pot	# of tubercle/pot	Infection severity	Biomass weight (g/pot)
Salale	0.0	7.5	0.0	2.1
Bulga-70	0.3	6.5	0.1	2.8
EH91016-5-1-1	0.7	1.3	0.2	0.6
HBP-B-10	1.0	12.5	0.3	5.8
HBP-C-21	1.0	3.3	0.3	1.9
BLB4342-1	1.3	4.3	0.4	2.0
EH96049-10	1.7	1.0	0.6	0.2
Hashenge	1.7	1.2	0.6	2.1
HBP-E-3	1.7	13.7	0.6	7.4
EH00005-3	1.7	3.0	0.6	4.9
EH00033-10-1	2.0	4.7	0.7	7.3
EH03007-1	2.0	5.0	0.7	3.6
EH03010- 1	2.0	8.0	0.7	3.7
EH00047-1-1	2.3	5.3	0.8	4.8
EH02177-1	2.3	6.7	0.8	4.0
EH02216-4	2.3	1.0	0.8	0.3
EH97031-2	2.7	4.5	0.9	1.3
Walki	3.0	6.0	1.0	7.9
EH02154-1	3.7	10.5	1.2	4.8
CS20DKxun-20V3	3.7	4.0	1.2	1.4
Coll59-99-1	3.7	2.7	1.2	2.1
EH02211-4	3.7	11.0	1.2	11.0
EH01044-1-1	3.7	4.0	1.2	6.7
EH95113-1	3.7	14.5	1.2	20.1
EH91007-2-1-4	4.0	4.7	1.3	4.1
EH00021-1	4.0	3.0	1.3	2.5
Coll168-00-1	4.0	1.0	1.3	2.9
EH03002-1	4.0	3.7	1.3	4.7
Local	4.0	5.0	1.3	4.9
EH00022-3	4.3	3.3	1.4	3.0
Coll021-01-10	4.3	3.0	1.4	2.0
HBP-C-13	4.3	3.0	1.4	4.7
EH43008-Ov3-3	4.3	1.3	1.2	1.7
Coll159-00-2	4.7	2.0	1.6	4.0
Wayu	4.7	8.5	1.6	6.2
Coll172-00-1	4.7	8.5	1.9	2.5
Dida	5.0	7.0	1.7	6.6
Degaga	5.0	4.4	1.7	3.2
E-03011-2	5.0	4.7	1.7	5.2
Coll192-00-1	5.0	6.0	1.7	2.4
EH99001-4	5.7	1.0	2.0	3.5
EH98106-1	6.3	10.7	2.1	6.8
EH02182-7	6.7	9.5	2.2	6.1
Mesay	6.7	1.0	2.2	9.8
Kasa	6.7	8.5	2.2	12.0
ILB4188	6.7	5.3	2.6	5.4
EH00101-2	7.0	4.7	2.3	3.9
EH02137-1	8.3	13.0	2.8	5.2
Dosha	9.3	11.2	3.1	2.3
Gebelicho	9.7	5.0	3.2	6.5
Cross-113-1	10.7	2.7	3.6	22.8
Coll218-00	13.0	11.5	4.3	7.7
Mean	4.22	5.68	1.42	5.02
LSD (0.05)	1.75	1.51	0.61	1.69

Faba bean reaction to crenate broomrape parasitism: The ANOVA showed statistically significant differences among faba bean genotype for mean growth variables as a consequence of crenate broomrape parasitism.

Dry biomass weight*:* Biomass weight of faba bean genotype ranged from 1.5 to 14.13 g per pot (Table 4). Highly significant ($p<0.01$) differences observed among the genotype in reacting against the crenate broomrape that the highest biomass weight of >13 g/pot obtained by genotype EH01044-1-1, Hashenge and EH02154-1 indicating their more relative resistance to the parasitism. Subsequently, the genotype with biomass weight of 6 to 13 g/pot grouped as moderately susceptible, while those scored 1.5 to 5.9 g/pot as highly susceptible. The results revealed that the breeding lines EH96049-10 and EH91016-5-1-1 recorded consistently the lowest shoots number and dry weight of crenate broomrape plant indicating their higher relative potential in reducing the parasite infection. While, variety Hashenge produced consistently the highest lateral roots length and biomass weight than the other genotype describing its higher relative resistance to the parasite infection. Supporting these results, Perez-de-Luque *et al*. (2010) reported that crop breeding lines displayed polygenic partial resistance to the parasitism that no one had showed immunity.

Lateral root length: Under the stress of crenate broomrape, mean lateral root lengths of faba bean genotype ranged from 17.17 to 227.50 cm per plant (Table 4). Significant ($p<0.05$) differences observed among the genotype, where the least root length of <30 cm/plant recorded from genotype Local, Mesay, Wayu, Walki, Bulga-70 and Coll59-99-1. In line with this research result, Tantawy *et al*. (2022) reported that the root length of faba bean plant was positively and significantly correlated with biological yield, but negatively and highly significantly correlated with the number and dry weight of broomrape. The intermediate resistance of Hashenge found against crenate broomrape, that confirm previous reports on the stability of the variety (Teklay Abebe *et al*., 2015; Mekonnen Misganaw, 2016; Lemma Diriba, 2023). Thus, one can be confident that developing partially resistant genotype could offer reliable defense against crenate broomrape, potentially improving faba bean yield and stability as reported by Perez-de-Luque *et al*. (2010).

Table 4. Mean biomass and lateral roots length of faba bean genotype inoculated with crenate broomrape

Genotype	Biomass weight (g)	Lateral roots length (cm)
EH01044-1-1	14.1	126.0
Hashenge	13.8	101.2
EH02154-1	13.8	66.7
EH98106-1	11.2	88.3
Cross-113-1	11.2	81.8
EH02177-1	10.9	65.7
Gebelicho	10.5	94.5
EH43008- Ov3-3	10.4	48.8
EH00021-1	9.3	57.5
EH00033-10-1	8.9	96.2
EH95113-1	8.8	69.7
EH02137-1	8.7	86.2
EH03007-1	8.4	59.8
EH03002-1	8.2	74.3
Dida	8.2	44.5
Salale	7.6	73.2
E-03011-2	7.6	85.3
EH91007-2-1-4	7.4	106.0
EH00005-3	7.3	52.3
Dosha	7.1	56.8
EH02216-4	7.0	44.5
EH99001-4	7.0	104.3
ILB4188	7.0	67.8
Coll021-01-10	7.0	54.5
EH02182-7	6.8	71.0
HBP-C-13	6.7	36.0
EH97031-2	6.7	53.5
CS20DKxun-20V3	6.7	53.2
Coll218-00	6.7	43.5
EH00101-2	6.6	82.5
Coll172-00-1	6.6	45.3
EH03010-1	6.6	67.0
Degaga	6.3	59.3
Coll159-00-2	6.3	79.0
Kasa	6.1	50.0
EH00022-3	5.7	57.2
HBP-B-10	5.7	31.0
Coll59-99-1	5.4	24.3
EH02211-4	5.1	227.5
Walki	5.1	21.2
HBP-C-21	5.1	66.7
Coll168-00-1	4.9	60.5
HBP-E-3	4.8	56.0
EH96049-10	4.7	55.8
BLB4342-1	4.6	108.5
EH91016-5-1-1	4.6	56.2
EH00047-1-1	4.5	59.2
Coll192-00-1	4.3	38.3
Mesay	4.1	17.2
Local	3.0	36.8
Wayu	2.6	20.2
Bulga-70	1.5	23.2
Mean	7.1	65.5
LSD (0.05)	3.1	12.41

Correlation among the host crop and parasite weed traits: Correlation analysis among faba bean and crenate broomrape plant parameters revealed positive and highly significant values between the parasitic plant shoots number and infection severity (0.99); and biomass weight and number of shoots (r=0.29), tubercles (0.29) as well as infection severity (r=0.32) as indicated in Table 5. In addition, the host crop lateral roots length also exhibited a strong positive (r=0.30) correlation with the crop plant biomass weight. Furthermore, the parasitic plant biomass weight correlated positively and significantly (r=0.18) with the host crop lateral roots length. Linke *et al*. (1991); Zaitoun *et al*. (1991) reported that the number of emerging *O. crenata* shoots negatively correlate with productivity of infected faba bean plants. The results also agree with the reports of Lemma Diriba (2023) that indicated the existence of considerable correlation between the parasite and host plant growth variables.

Table 5. Correlation among crenate broomrape and faba bean traits

	[1]CBNS	IS	CBNT	CBBW	FLRL
IS	0.991**	-	-	-	-
CBNT	0.147	0.148	-	-	-
CBBW	0.294**	0.287**	0.319**	-	-
FLRL	0.034	0.040	0.096	0.181*	-
FBW	0.153	0.142	0.043	0.117	0.302**

[1]CBNS= crenate broomrape number of shoots, IS= infection severity, CBNT= crenate broomrape number of tubercles, CBBW= crenate broomrape biomass weight, FLRL= faba bean lateral roots length, FBW= faba bean biomass weight, **. Correlation is highly significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

Conclusion

The study demonstrated considerable variation among faba bean genotype in response to crenate broomrape parasitism under glasshouse conditions. Genotype such as ‘Hashenge’, EH96049-10, and EH91016-5-1-1 consistently exhibited lower infection severity, fewer crenate broomrape shoots and tubercles, and reduced parasitic biomass, indicating moderate resistance and the presence of promising defense-related traits. Conversely, susceptible genotype showed higher parasite emergence and biomass, and severe host damage, highlighting the potential yield losses caused by this parasitic weed. Faba bean biomass and lateral root development were significantly affected by crenate broomrape infection, with lower weight of the crop plant biomass generally associated with higher parasite establishment. Correlation analysis revealed significant relationships between host biomass and parasite growth. Overall, the findings emphasize the potential of partially resistant faba bean genotype as a sustainable strategy to manage crenate broomrape and mitigate yield losses. The identified genotype, particularly those combining high biomass with reduced parasite susceptibility, can serve as valuable candidates for breeding programs aimed at improving faba bean productivity and stability in crenate broomrape infested regions of Ethiopia.

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