



UNIVERSIDAD DE CÓRDOBA

**Programa de Doctorado en Ingeniería Agraria, Alimentaria, Forestal y
del Desarrollo Rural Sostenible**

TESIS DOCTORAL

**Nuevos Genes y Herramientas para el Manejo de Malas Hierbas
en Habas**

Novel Genes and Tools for Weeds Management in Faba bean

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FOUAD MAALOUF

Marzo de 2022

TITULO: *NOVEL GENES AND TOOLS FOR WEEDS MANAGEMENT IN FABABEAN*

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**Doctorate Program of Agricultural, Food, Forestry, and Sustainable
Rural Development Engineering**

PhD THESIS

Novel Genes and Tools for Weeds Management in Faba bean

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March 2022



**TÍTULO DE LA TESIS: NOVEL GENES AND TOOLS FOR WEEDS MANAGEMENT
IN FABA BEAN**

DOCTORANDO/A: Lynn Abou-Khater

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(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

Los trabajos de campo de esta tesis doctoral se han desarrollado entre 2014 y 2019 en las estaciones experimentales de ICARDA en Marruecos y Líbano. La doctorando ha realizado estancias breves en Córdoba, cumpliendo con todos los requisitos del programa de doctorado y tutela académica. Entre 2020 y 2021 se han analizado los datos y redactado los capítulos resultantes, siempre en comunicación directa y fluida con sus dos codirectores, uno de los cuales estaba en Córdoba y el otro en el Líbano. Como resultado, los cuatro capítulos de la tesis han sido ya publicados o están en prensa siendo en todos ellos la doctoranda primera autora:

- Capítulo 1: Abou-Khater et al. (2022). Status of faba bean (*Vicia faba* L.) in the Mediterranean and East African countries. In U.C. Jha., H. Nayyar., S. Kumar., K. Siddique (Eds.). *Developing Climate-resilient Food and Fodder Legumes*. Springer-Nature, en prensa
- Capítulo 2: Abou-Khater et al. (2021). Identification of tolerance to metribuzin and imazethaprid herbicides in faba bean. *Crop Science* 61: 2593-2611. doi:10.1002/csc2.20474, IF₂₀₂₀=2.32, Ranking 30/91 (*Agronomy*) Q2.
- Capítulo 3: Abou-Khater et al. (2022). Genomic regions associated with herbicide tolerance in a worldwide faba bean (*Vicia faba* L.) collection. *Scientific Reports* 12:158, <https://doi.org/10.1038.s41598-021-03861-0>, IF₂₀₂₀=4.38, Ranking 17/73 (*Multid. Sciences*) Q1
- Capítulo 4: Abou-Khater et al. (2022). Adaptability and stability of faba bean (*Vicia faba* L.) accessions under diverse environments and herbicide treatments. *Plants* 11: 251. <https://doi.org/10.3390/plants11030251>, IF₂₀₂₀=3.94, Ranking 47/235 (*Plant Sciences*), Q1

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TÍTULO DE LA TESIS:
NOVEL GENES AND TOOLS FOR WEEDS MANAGEMENT IN FABA BEAN

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ABSTRACT

Weeds are one of the greatest threats to faba bean (*Vicia faba* L.) production and herbicide application is known to be the most efficient weed control method. However, the susceptibility of the current cultivars to post emergence herbicide applications impose a limitation for weed control in faba bean. Therefore, the deployment of postemergence herbicide tolerance is desirable in faba bean. To address this, a set of 130 faba bean accessions were screened for their response to the recommended dosages of two herbicides, metribuzin at 250 g a.i. ha⁻¹ and imazethapyr at 75 g a.i. ha⁻¹ at Marchouch and Terbol stations during four seasons between 2014 and 2018. The herbicide damage score along with other phenological and yield related traits were recorded and analyzed to evaluate the effects of post emergence herbicide application on the tested accessions and to select the herbicide tolerant ones. Tolerance to metribuzin and imazethapyr in eight faba bean accessions was confirmed as no significant reduction in grain yield resulted from the herbicide application.

Moreover, in the aim to identify superior and broad adapted herbicide tolerant faba bean genotypes and to identify the most discriminating environments for herbicide screening, 37 accessions with different levels of tolerance to the recommended dosages of metribuzin and imazethapyr were selected to further evaluate the performance and stability of their plant height and grain yield across different environments (location x season x herbicide treatment). Data collected at Marchouch and Terbol during four seasons between 2014 and 2019 were used and the evaluation was based on the following stability parameters: cultivar superiority, static stability, Wricke's eco-valence and Finlay and Wilkinson's regression model. Differences observed in the genotypes ranking suggest that the evaluation of performance and stability of herbicide tolerant faba beans should be based on a combination of stability parameters. Genotype * environment biplot analysis indicated that the environments representing the metribuzin treatment at Marchouch 2014–2015 and the non-treated treatment at Terbol 2018–2019 are the ideal environments for evaluating faba bean genotypes. Biplots showed also that the metribuzin and imazethapyr tolerant accession IG12983 is the ideal genotype as he showed good and stable plant height and grain yield performance across the environments.

Finally, to identify molecular markers associated with key genes imparting tolerance to herbicides the same set of accessions phenotyped before was genotyped using genotyping by sequencing (GBS). The GBS yield 10,794 high quality single nucleotide polymorphisms (SNPs).

10 and 14 SNPs highly associated with phenological and yield related traits under herbicide treatments were identified after conducting Single-trait and Multi-trait Genome Wide Association Studies (GWAS) respectively. Genomic sequences containing herbicide tolerance associated SNPs were aligned against the NCBI database using BLASTX tool and default parameters to annotate candidate genes underlying the causal variants. SNPs from acidic endochitinase, LRR receptor-like serine/threonine-protein kinase RCH1, probable serine/threonine-protein kinase NAK, malate dehydrogenase, photosystem I core protein PsaA and MYB-related protein P-like were significantly associated with herbicide tolerance traits. The identified SNPs will facilitate and fasten the efficient and effective development of herbicide tolerant cultivars and can be used for introgressing herbicide tolerance into desired agronomic background.

RESUMEN

Las malas hierbas se consideran como mayor amenaza para la producción de las habas (*Vicia faba* L.) ya que no se dispone de métodos efectivos de control adecuados particularmente en el estado de postemergencia. En un intento de mejorar la tolerancia a los herbicidas de postemergencia más comunes, se evaluó durante cuatro años la respuesta de una colección de 130 accesiones de habas a las dosis recomendadas de metribuzin (250 g i.a. ha⁻¹) e imazetapir (75 g i.a. ha⁻¹) en condiciones de campo en las estaciones experimentales de Marchouch (Marruecos) y Terbol (Líbano). Estos trabajos han permitido confirmar la tolerancia de ocho accesiones a metribuzin y a imazetapir. Asimismo, se ha estudiado la estabilidad de componentes del rendimiento de 37 accesiones de habas con diferentes niveles de tolerancia a las dosis recomendadas de ambos herbicidas en diferentes ambientes (localidad x año x tratamiento con herbicida). Los resultados mostraron que la estabilidad de la respuesta en habas tolerantes a herbicidas se basa en una combinación de efectos. Los análisis de interacción Genotipo*Ambiente mostraron el tratamiento con metribuzin en Marchouch 2014-2015 y el control sin herbicida en Terbol 2018-2019 son los ideales para evaluar los genotipos de habas con tolerancia a herbicidas. La accesión IG12983 tolerante a ambos herbicidas mostró una altura de planta y rendimiento de grano altos y estables en todos los ambientes.

Finalmente, la colección de habas fue genotipada por secuenciación (Genotyping by Sequencing “GBS”), resultando en la identificación de 10.794 polimorfismos de un solo nucleótido (SNP) de alta calidad. El análisis de asociación (Genome Wide Association “GWAS”) permitió la identificación de 10 y 14 SNP altamente asociados con tolerancia a los herbicidas, según se analizara por un solo o por múltiples caracteres, respectivamente. Las secuencias genómicas que contenían SNP asociados con la tolerancia a los herbicidas se alinearon con la base de datos del NCBI utilizando y la herramienta BLASTX y parámetros predeterminados para anotar los genes candidatos subyacentes a las variantes causales. Los SNP de la endoquitinasa ácida, la serina/treonina-proteína quinasa RCH1 similar al receptor LRR, la probable serina/treonina-proteína quinasa NAK, la malato deshidrogenasa, la proteína PsaA del núcleo del fotosistema I y la “MYB”- relacionada con proteína “P-like” se asociaron significativamente con los caracteres de tolerancia a herbicidas. Estos SNP identificados permitirán acelerar el desarrollo eficiente y eficaz de cultivares tolerantes a herbicidas.

TABLE OF CONTENTS

CHAPTER I.....	1
General Introduction.....	1
1. Abstract.....	1
2. Thesis objectives.....	2
CHAPTER II.....	3
Identification of tolerance to metribuzin and imazethapyr herbicides in faba bean.....	3
3. Materials and Methods.....	6
3.1. Materials.....	6
3.2. Site-Season Experiments.....	8
3.3. Preliminary screenings.....	9
3.4. Validation of the results.....	10
3.5. Final validation against dosages.....	10
3.6. Observations needed.....	10
3.7. Statistical analysis.....	11
4. Results.....	12
4.1. Herbicide damage score.....	12
4.2. Crop phenology.....	15
4.3. Plant architecture.....	16
4.4. Yield and yield components.....	16
4.5. Herbicide reduction indexes.....	18
4.6. Selection for tolerant accessions.....	20
5. Discussion.....	22
5.1. Response to herbicide treatments.....	22
5.2. Effects of herbicide on crop phenology.....	23
5.3. Effect of herbicide on plant architecture.....	23
5.4. Effect of herbicide on yield components.....	24
5.5. Selection for herbicide tolerance.....	24

6. Conclusions.....	24
7. References.....	25
CHAPTER III	31
Adaptability and Stability of Faba Bean (<i>Vicia faba</i> L.) Accessions under Diverse Environments and Herbicide Treatments	31
1. Abstract.....	31
2. Keywords	32
3. Introduction.....	32
4. Materials and Methods.....	33
4.1. Materials	33
4.2 Experiments	33
4.3 Recorded Traits.....	36
4.4 Statistical Analysis	36
5 Results.....	38
5.1 Phenological traits	38
5.2 Plant Height	40
5.3 Grain Yield	45
5.4 GGE Analysis	48
6 Discussion.....	52
7 Conclusions.....	56
8 References.....	57
9 Supplementary Materials	64
CHAPTER IV	66
Genomic regions associated with herbicide tolerance in a worldwide faba bean (<i>Vicia faba</i> L.) collection.....	66
1. Abstract.....	66
2. Introduction.....	67
3. Materials and Methods.....	68
3.1. Plant Materials	68
3.2. Experiments	69

3.3	Phenotyping for herbicide tolerance.....	70
3.4	DNA extraction and genome by sequencing analysis	71
3.5	Statistical analysis of phenotyping data.....	71
3.6	Genome-wide association analysis.....	72
4.	Results.....	72
4.1.	Phenotyping	72
4.2.	Genotyping and population structure	77
4.3.	GWAS and annotation analyses	78
5.	Discussion	82
6.	Conclusions.....	84
7.	References.....	85
8.	Supplementary Materials	91
CHAPTER V	98
General Conclusions	98

CHAPTER I

General Introduction

This chapter is based on:

Abou-Khater, L., Maalouf, F., and Rubiales, D. (inpress). Status of faba bean (*Vicia faba* L.) in the Mediterranean and East African countries. In U.C. Jha., H. Nayyar., S. Kumar., K. Siddique (Eds.). *Developing Climate-resilient Food and Fodder Legumes*. Springer Nature.

1. Abstract

Faba bean (*Vicia faba* L.) was first domesticated in the Fertile Crescent of the Near East. It ranks fourth among the cool season food legumes and is now grown in more than 66 countries. Faba bean is a versatile crop; besides being an important source of protein for food and feed, it offers many services for the ecosystem. The Mediterranean and East African countries account for nearly 32% of the global faba bean production. However, the interest in growing faba bean in these countries does not seem to meet their demand even though it is a crop of great economic and social importance there and it is well adapted to Mediterranean-like environments and to the Highlands of sub-tropical environments. Numerous factors affect the production of faba bean in the Mediterranean and East African countries including the limited financial support that is posing a challenge to the crop improvement for the major production and adoption constraints such as the biotic and abiotic stresses and the antinutritional components. While major achievements have been made towards the development of faba bean varieties having desired traits, there is still a lot of work to be done. The high level of genetic diversity in faba bean accessions and the available molecular markers will considerably help tighten the gap between the production and the demand in the Mediterranean and East African region.

2. Thesis objectives

The three main objectives of the Ph.D. thesis are:

- (i) Identify faba bean lines with tolerance to post-emergence application of two herbicides namely metribuzin and imazethapyr, to assess herbicide effect on different phenological, agronomic, and yield traits in faba bean and to evaluate the efficiency of the visual scoring of damages when screening faba bean lines for herbicide tolerance (Chapter II).
- (ii) Evaluate the performance and yield stability of herbicide tolerant faba bean lines under different environments with combined effect of herbicide treatment, location, and season and to identify the best environments to screen faba bean for herbicide tolerance (Chapter III).
- (iii) Identify candidate loci significantly associated with tolerance to the post emergence application of metribuzin and imazethapyr under different environments using GWAS and to identify associated SNP markers that can be used for introgressing such traits into desired agronomic background (Chapter IV).

CHAPTER II

Identification of tolerance to metribuzin and imazethapyr herbicides in faba bean

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1. Abstract

Weeds cause serious constraint to faba bean (*Vicia faba*L.) productivity. Broad weed control is hampered by the availability of postemergence herbicides to control them, as the current faba bean cultivars are highly susceptible to them. Therefore, the deployment of postemergence herbicide tolerance is desirable in faba bean. To address this, 130 accessions were screened for their response in mature plants under field conditions to the recommended dosage of two herbicides, metribuzin at 250 g a.i. ha⁻¹ and imazethapyr at 75 g a.i. ha⁻¹ at Marchouch and Terbol stations. The recorded herbicide damage score (HDS) varied from 1 (no visual damage) to 5 (full damage with death of more than 50% of plants) at both locations. Low but highly significant ($p < .01$) and positive correlation (+0.26) was obtained between the recorded HDS at both locations. Both herbicides significantly delayed flowering and maturity time occurrence, reduced plant height and grain yield, and increased number of branches. Reduction index (RI) correlated positively with HDS score at Terbol station in different seasons and at Marchouch in 2016–2017 seasons. Eleven tolerant accessions were identified and further evaluated to 1×, 1.5×, and 2× of recommended dose of both herbicides. The results indicated that the harmful effect of herbicides on grain yield reduction intensified from 13.4 to 27.2% and from -7.6 to 1.8% as the dose of metribuzin and imazethapyr increased respectively from 250 to 500 g a.i. ha⁻¹ and from 75 to 150 g a.i. ha⁻¹. Tolerance to

metribuzin and imazethapyr in eight faba bean accessions was confirmed with no significant reduction in grain yield.

2. Introduction

Faba bean (*Vicia faba* L.) is one of the most important cool-season grain legumes widely grown on 2.5 million ha area in 38 countries (www.faostat.fao.org). It contributes to sustainable agriculture and ecosystem services by enriching the soil with nitrogen (N), improving yield of subsequent cereal crop, and diversifying the cropping systems (Jensen et al., 2010; Ruisi et al., 2017). Being a partially allogamous crop, faba bean plays a key role in conserving the insect pollinators that transfer the cross-pollen and improve the seed set (Marzinzig et al., 2018). In addition to its ecosystem services, faba bean is a highly nutritious legume because of the high protein content of its seeds (Crépon et al., 2010). However, its cultivation remains stagnant over the last 20 yr in western Asia and northern Africa (WANA) where cereal monoculture represents >90% of the agricultural land (www.faostat.fao.org). This is because of various biotic and abiotic stresses and agricultural practices such as the limitation of efficient postemergence herbicides and the dependence in manual or mechanical weeding. Inclusion of faba bean in cereal cropping systems requires many factors such as developing new cultivars that are amenable to modern agricultural practices, including mechanized harvesting and chemical weed control, and cultivars resistant to pests and diseases and tolerant to the different abiotic stresses. The major limiting factors of faba bean production in the WANA region are parasitic and nonparasitic weeds (Maalouf et al., 2016a). Faba bean has low competitive ability with annual weeds commonly encountered in faba bean fields in the region because of its slow initial growth, which favors the emergence and growth of annual weeds before the ground is covered by the crop canopy (Frenda et al., 2013; GRDC, 2017a). Many weeds compete for nutrients, light, and moisture with the growing faba bean plants. But the effect of weeds is not limited to competition only, they also act as alternative hosts for many pathogens, viruses, and insect pests that may lower grain yield and seed quality (Parihar et al., 2017). Weeds such as white mustard (*Sinapis alba* L.), *Eruca sativa* Mill., common amaranth (*Amaranthus retroflexus* L.), lamb's-quarters (*Chenopodium album* L.), butterweed (*Erigeron canadensis* L.), and common nightshade (*Solanum nigrum* L.) might also show allelopathic effect through releasing chemical compounds that suppress the growth of faba bean and other grain legume plants (El-Masry et al., 2015; Klingman et al., 1982; Marinov-Serafimov, 2010; Messiha et al., 2018). The presence of weeds in faba bean fields also hinders clean harvesting of the crop, as it usually matures earlier than the weeds (GRDC, 2017a). Economic losses as a result of weeds in farmers' fields can vary

from negligible to a complete crop loss. Manual weeding in the WANA region is expensive and can cost ~US\$600 ha⁻¹. In addition to standard weeds, parasitic weeds, namely root parasitic broomrapes (*Orobancha* spp.) and stem parasitic dodders (*Cuscuta* spp.) also affect faba bean in many production regions (Rubiales & Fernández-Aparicio, 2012). Broomrapes can be particularly harmful, causing complete loss of faba bean crop, being widely distributed in the Mediterranean basin (Fernández-Aparicio et al., 2016; Maalouf & Baum, 2015). The integrated weed management practices combining both chemical and nonchemical methods, such as biological weed control, hand weeding, mechanical weeding, crop rotations, tillage, soil solarization, and herbicide applications, are recommended for effective weed control in faba bean (Burn-Side et al., 1998; Singh & Singh, 2012). In conventional agriculture, herbicide treatment still appears as the most efficient, less time consuming, and less costly than other methods because of the high cost of labor in both developed and developing countries and high energy cost for mechanical weed control (García De Arevalo et al., 1992; Gressel, 2000). Metribuzin and imazethapyr are commonly available chemical herbicides that can control the majority of weeds. However, like other legumes, faba bean cultivars are sensitive to these herbicides, with severe phytotoxicity and negative effect on the crop cycle and crop production as many scientists reported a delay in the flowering and maturity time (Gupta et al., 2017; Jefferies et al., 2016; Sajja et al., 2015; Taran et al., 2013) and a reduction of the height (Sajja et al., 2015; Sharma et al., 2016, 2018), yield, and yield components of different legume crops treated with metribuzin and imazethapyr (Sharma et al., 2016, 2018; Taran et al., 2010). Metribuzin belongs to Triazines chemical group, which disrupts electron transfer through binding to the D1 protein of the photosystem II complex in chloroplast thylakoid membranes (Senseman, 2007). Metribuzin can control dodder as well as other annual weeds (GRDC, 2017b) and has been recommended for managing weeds in legume crops in many countries (Datta et al., 2009). Imazethapyr is a systemic herbicide that belongs to IMI class of herbicides (Imidazole) that control weeds by reducing the level of branched-chain amino acids— isoleucine, leucine, and valine—through the inhibition of acetolactate synthase (ALS), an enzyme common to the biosynthesis of these amino acids. Imazethapyr can control broomrape (Dor et al., 2017; García-Torres & López-Granados, 1991; Rubiales & Fernández-Aparicio, 2012; Tanet et al., 2005) and annual weeds (Cantwell et al., 1989). In order to make legumes amenable to herbicide application and expand their cultivation in many production regions, tolerance to herbicides through mutagenesis and germplasm screening has been explored with examples of metribuzin tolerance in faba bean (Maalouf et al., 2016b), soybean [*Glycine max* (L.) Merr.] (Hartwig, 1987), lupin

(*Lupinus albus* L.) (Si et al., 2009), and lentil (*Lens culinaris* Medik.) (McMurray et al., 2019) and imazethapyr tolerance in chickpea (*Cicer arietinum* L.) (Chaturvedi et al., 2014; Gaur et al., 2013), lentil (Sharma et al., 2016, 2018; Singh et al., 2016; Slinkard et al., 2007), and field pea (*Pisum sativum* L.) (Hanson & Thill, 2001). However, such studies are preliminary in nature based on a single location screening of limited number of germplasms in faba bean. Therefore, the present study was carried out to screen a large number of faba bean germplasms at two different locations over four seasons for identification of stable tolerance to metribuzin and imazethapyr; to assess herbicide effect on different phenological, agronomic, and yield traits in faba bean; and to evaluate the efficiency of the visual scoring of damages when screening faba bean germplasm to herbicide tolerance.

3. Materials and Methods

3.1. Materials

A subset of 130 faba bean accessions belonging to the four different subspecies (9 *paucijuga*, 62 *equina*, 42 *major*, and 17 *minor*) were included in the present study along with checks. These accessions (Table 1) are pure lines obtained after at least three self-pollinated generations under insect proof and derived from landraces collected from 35 countries and from breeding materials with wide range of genetic diversity assessed by simple sequence repeat markers (Maalouf et al., 2019). The seeds used in the current experiments are sourced from the seed multiplication conducted each year under insect-proof cages in order to avoid cross pollination and ensure the purity of the evaluated accessions.

Table 1. *Subspecies and country of origin of the different accessions tested for herbicide tolerance.*

Subspecies	Country	Accessions	Country	Accessions
<i>Vicia faba</i> var. <i>equina</i>	Afghanistan	IG11726	Morocco	IG13771
	Algeria	IG11561, IG12110	Netherlands	FB1482
	Bulgaria	VF283	Pakistan	IG11527, IG108537
	Canada	IG13906, IG74363, IG130693	Peru	IG12135, IG14196, VF845, FB2047
	China	IG99328, IG124479, IG132194	Portugal	IG14209
	Cyprus	IG13468	Russia	VF324
	Ecuador	IG126172, IG126202	Spain	VF367, VF916, VF972, VF989, VF683, VF729

	Egypt	VF345, VF512, VF522	VF510, VF513,	Sudan		IG13945
	Ethiopia	IG11742, IG11908, IG14026, VF268		Switzerland		FB1682, FB1709
	France	FB310, FB1564, FB2077, FB2509, FB2528, FB2583		Syrian Republic	Arab	IG72481
	Germany	IG130496		Tunisia		VF545
	ICARDA	IG99664, IG101949, IG104374, IG106331, IG106453		Turkey		VF351
	Iraq	IG11982		Ukraine		IG130402
	Italy	IG14212, IG130520		United Kingdom		FB1197, FB1783
<i>Vicia faba</i> var. <i>major</i>	Afghanistan	VF420		Morocco		IG100096
	Bangladesh	IG14163		Netherlands		FB1216
	Canada	IG11843		Poland		FB199
	China	IG12158		Portugal		IG70584, IG99419
	Cyprus	IG13513, IG13530, IG13547		Russia		VF339
	Egypt	VF481		Spain		VF878, VF887, VF955, VF963, VF703, VF944, VF950
	Ethiopia	VF270, FB2648		Switzerland		FB1720
	France	FB2041, FB2515, FB2574		Syrian Republic	Arab	IG70622
	Germany	FB1512		Tunisia		VF544, IG12983
	ICARDA ^a	IG104985, IG105789, IG105844, IG103102, IG104421, IG104526, IG104821		Turkey		IG11388
	Iraq	IG11232		United Kingdom		FB1213
<i>Vicia faba</i> var. <i>minor</i>	Canada	IG74341		France		FB2568, FB2601, FB1165
	Ecuador	IG124721		Nepal		IG115303
	Ethiopia	IG12659, VF419		Spain		VF674
	Germany	FB1631		Syrian Republic	Arab	IG13008, IG13958, IG72498
	ICARDA ^a	IG103043		Ukraine		IG130407
	Italy	IG13231		Unknown		VF260
<i>Vicia faba</i> var. <i>paucijuga</i>	ICARDA ^a	IG104039		ICARDA ^a		IG104082
	Russia	VF335		Czech Republic		VF301

	Nepal	IG115213	ICARDA	IG106984
	China	IG126166	United Kingdom	VF626, VF810

^a Breeding lines.

3.2. Site-Season Experiments

In total, five site–seasons experiments were conducted at two ICARDA experimental stations, namely Terbol (35.98° N, 33.88° E, 890 m asl) in Bekaa Valley of Lebanon during three consecutive seasons from 2015–2016 to 2017–2018, and Marchouch (33.5581° N 6.6930°W, 255 m asl) in Morocco during the 2014–2015 and 2016–2017 seasons. Terbol station, where the soil is deep and rich clay loam is characterized by cool and high rainfall winter and moderate and wet spring. Climatic data, described in Figure 1, indicated high rainfall between December and March and a wet and warm spring in all seasons. The highest rainfall and lowest temperatures were observed in 2016–2017 while the highest temperatures were observed in 2017–2018. Marchouch station, in which the soil is Vertisol and mostly silty clay, is characterized by semiarid environment. Figure 1 indicates high rainfall winter and dry spring in both seasons and a warm 2016–2017 season. Supplemental irrigation of 30 mm was provided at all site–seasons during dry spells periods expect for the experiment conducted in 2014–2015 at Marchouch. These site–seasons experiments were sown in rotation with cereals, either durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren] or bread wheat (*Triticum aestivum* L.) in late November at Terbol and in mid-December at Marchouch and harvested in late May at both locations. Good agronomic practices were adopted to raise a successful crop by adding 15-15-15 of granulated NPK at 250 kg ha⁻¹ and spraying lambda-cyhalothrin at 40 g a.i. ha⁻¹ to control sitona, imidaclopid at 160 g a.i. ha⁻¹ to control aphids, and a combination of azoxystrobin and difenoconazole at 72.8 and 45.6 g a.i. ha⁻¹, respectively, to control foliar diseases. Weeds were controlled by pre-emergence application of pendimethalin at 1,200 g a.i. ha⁻¹ followed by manual weeding to avoid weed competition. The major weeds found in our fields at both locations were bean broomrape (*Orobanche crenata* Fors.), dodder (*Cuscuta campestris* Yunck.), rapeseed (*Brassica napus* L.), bindweed (*Convolvulus arvensis* L.), and narrow-leaved weeds.

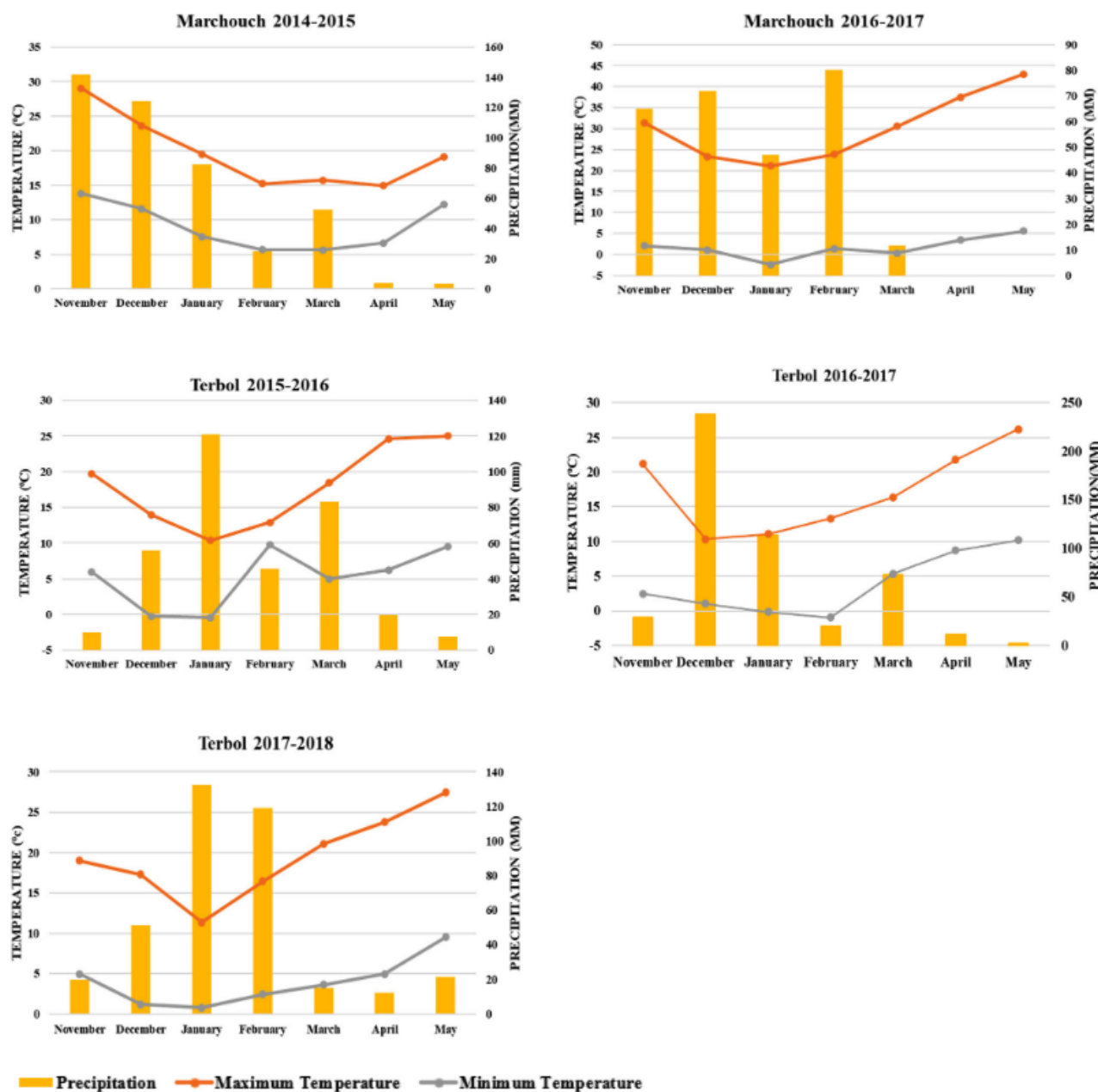


Figure 1. Maximum temperature (°C), minimum temperature (°C), and precipitation (mm) at Marchouch and Terbol during different planting seasons.

3.3. Preliminary screenings

Preliminary screening of faba bean germplasm was performed at Marchouch and Terbol stations by spraying the recommended dosages of metribuzin at 250 g a.i. ha⁻¹ (T1) and imazethapyr at 75 g a.i. ha⁻¹ (T2) at the inflorescence emergence stage (BBCH code 50) (Lancashire et al., 1991) along with the untreated plots control, C). Each accession was planted in a 2-m-long, one-row plot with 0.5 m spacing between rows. At Marchouch, the tested accessions were evaluated in unbalanced

block design with the three herbicide treatments during 2014–2015. The tested accessions were planted in an augmented design with randomized blocks. In addition to tested accessions, each block contains three replicated checks (two *major* types, FLIP86-98FB, ILB1814, and one *equina* type BPL710) to monitor the experimental errors. At Terbol, the tested accessions with four additional accessions having diverse genetic background, (Flip86-98FB is *major* type, HBP/SOC/2003 and ILB365 are *equina*, and NA112 is *paucijuga*) were screened in alpha lattice design with two replications and with same herbicide treatments during 2015–2016.

3.4. Validation of the results

Based on the results of preliminary screening, selected accessions were screened for validation of the results. At Marchouch, 40 accessions (35 showing low visual damage to herbicides and no significant reduction in plant height and grain yield under one or both the herbicides, five showing severe damage) were further evaluated in unbalanced block design with two replications against the same three herbicide treatments (T1, T2, and C) during 2016–2017 season. Each plot was planted in a 2-m-long, two-row plots maintaining 0.5 m distance between the rows. At Terbol, 26 accessions showing low visual damage and no significant reduction in grain yield and one susceptible accession, NA112, were evaluated again during 2016–2017 following the same design with three replications.

3.5. Final validation against dosages

Eight accessions were selected at Terbol and three were selected at both locations in 2016–2017 season showing low visual damage and no significant reduction in grain yield were further evaluated against higher dosages (1×, 1.5×, and 2× of recommended rate) of metribuzin and imazethapyr at Terbol during 2017–2018. Three treatments each of metribuzin (T1 = 250 g a.i. ha⁻¹, T3 = 375 g a.i. ha⁻¹, and T4 = 500 g a.i. ha⁻¹) and imazethapyr (T2 = 75 g a.i. ha⁻¹, T5 = 112.5 g a.i. ha⁻¹, and T6 = 150 g a.i. ha⁻¹) dosages along with control (C) were evaluated in an unbalanced block design with three replications.

3.6. Observations needed

Herbicide damage score (HDS) was recorded twice as a preliminary observation using a 1-to-5 scale (Table 2) during flowering (BBCH code 60, HDS1) and pod development (BBCH code 70, HDS2) stages (Lancashire et al., 1991). The purpose of HDS2 was to monitor the regeneration ability of each accession. Observations on days to flowering (DFLR) at 50% of flowered plants, days to maturity (DMAT) at 50% of matured plants, plant height (PLHT) as average of three plants,

number of branches per plant (BRPLT) as average of three plants, number of seeds per plant (SNPLT), grain yield per plant (GYPLT) as average of three plants, and 100-seed weight (HSW) as average of three plants were recorded as described in the published ontology by Maalouf (2018). The traits DFLR, DMAT, PLHT, and GYPLT were assessed for T1, T2, and C in all seasons at Terbol and Marchouch. The traits PNPLT and SNPLT were assessed for T1, T2, and C at Terbol in all seasons and for T1 and T2 at Marchouch 2014–2015. Traits BRPLT and HSW were assessed for T1, T2; and C at Terbol 2017–2018. The effect of herbicide treatments on different accessions based on the HDS was assessed by estimating the reduction in grain yield and plant height at Terbol during 2015–2016 and 2016–2017. The reduction index (RI) of tolerance was estimated as follows:

$$RI\% = \left(1 - \frac{\bar{T}}{\bar{C}}\right) * 100$$

where RI% is the reduction index of tolerance that represents the reduction in traits after herbicide treatment, \bar{T} is the average of plots treated with herbicide (metribuzin or imazethapyr), and \bar{C} is the mean of accessions under untreated conditions.

Since the yield reduction is the most important trait to rely on when selecting tolerant accessions, correlation between HDS and RI of grain yield per plant (RI_{GY}) was calculated to evaluate the relationship between these two variables and see if the assessment of the visual symptoms could be a reliable indicator for herbicide tolerance and replace the yield assessment that is very laborious. The selection of tolerant accessions was based mainly on RI_{GY} of each accession.

3.7. Statistical analysis

The spatial statistical model was applied for all quantitative data using the automatic spatial variance analysis using incomplete block design of Genstat 19 edition (Goedhart & Thissen, 2018) within environments (for each site–season independently); the fixed factors were genotypes and treatments, while random factors were plots, blocks, and replications. Variations among accessions, treatments, and accessions \times treatments interaction were assessed in terms of p values (probability of observing more extreme data that can be observed under the hypothesis of no genotypic variation) using the Wald statistic. The best linear phenotypic estimates were estimated for each treatment and accessions within the treatments in each year and site separately. Spearman correlation analysis was performed between HDS scores recorded at both preliminary screening locations and between HDS scores and RIs to assess the level of similarity of scoring at both locations and to evaluate the efficiency of visual scoring method as compared with the RIs. Ordinal

regression was performed to predict the behavior of HDS scores with the estimated RIs either in plant height and or in grain yield.

Table 2. Description of the damages observed in the treated plants for each herbicide damage score (HDS)

HDS	Description
1	No damage observed; Normal phytosanitary status; Normal and very good vegetative growth
2	Very light damage observed; Very few leaf burnings; Very good phytosanitary status
3	A clear moderate damage observed; Stunting in growth with high yellowing; Necrosis on leaves
4	A high damage was observed and death of <50% of plants; Severe yellowing, leaf and stem burning with high deformations; Very weak vegetative growth and stunted plants
5	Severe damage and death of >50% of plants; High deformations and burnings; High reduction of plant's biomass; Overall yellowing was detected

4. Results

4.1. Herbicide damage score

Faba bean accessions treated with metribuzin and imazethapyr were given a 1-to-5 score (Table 2) at the first HDS (HDS1) stage, indicating a wide range of genotypic variation among the tested accessions across locations and seasons. Most of the accessions treated with metribuzin showed leaf burnings, necrosis, yellowing, and reduced growth. The highly susceptible accessions were completely damaged with total burning and ultimately death of all plants. Most of the accessions treated with imazethapyr showed leaf yellowing, leaf size narrowing, growth reduction, and stem deformation at the apical meristem.

During 2014–2015 season at Marchouch, the second HDS (HDS2) was recorded only after 4 wk of metribuzin and imazethapyr treatments. It varied from 1 to 5, showing wide range of genotypic variation. Among the accessions treated with imazethapyr, 2% of the accessions showed very low damage (HDS2 = 1–2), 26% showed moderate damage (HDS2 = 3), and remaining ones showed high or very high damage (HDS2 = 4–5). In case of metribuzin, 10% of the accessions showed very low damage (HDS2 = 1–2), 47% showed moderate damage (HDS2 = 3), and remaining showed high or very high damage (HDS2 = 4–5) (Figure 2c).

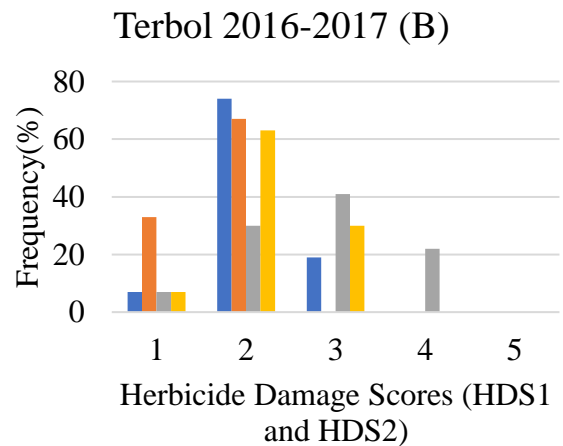
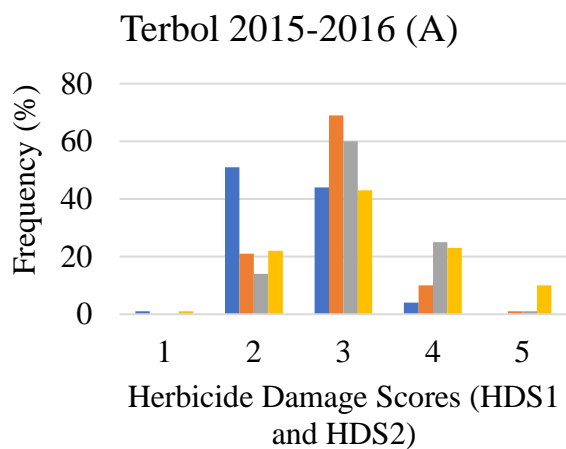
The results indicated that 35 accessions showed low or moderate damage (HDS2 = 1–3) to one or both the herbicides and were selected for further evaluation during 2016–2017 season at Marchouch. The HDS1 score was 2 or 3 for 88% of the accessions after imazethapyr and 72% of the accessions after metribuzin treatment, whereas the HDS2 was 2 or 3 for 53% accessions after

imazethapyr and 77% after metribuzin, showing aggravation of symptoms rather than recovery after imazethapyr and metribuzin treatment (Figure 2d).

The preliminary screening performed at Terbol during 2015–2016 also showed a wide range of variation (1–5) at HDS1. Among the evaluated accessions, 52% showed low (HDS1 = 1–2), 44% showed moderate (HDS1 = 3) and remaining ones showed high or very high (HDS1 = 4–5) HDS score when treated with Imazethapyr. While 14% of the accessions showed very low (HDS1 = 2), 60% showed moderate (HDS1 = 3) and remaining showed high or very high (HDS1 = 4–5) damage when treated with metribuzin. The herbicide damage HDS2 recorded after 4 wk of the treatments revealed that approximately 44 and 20% of the accessions recovered from the metribuzin and imazethapyr treatments respectively. The HDS2 score after imazethapyr treatment was low (2) for 21% of the accessions, moderate (3) for 69% accessions, and high or very high (4–5) for the remaining accessions. The accessions showing severe damage in the first score continued with the same levels of damage in the second score (HDS2 = 4–5). Concerning metribuzin treatment, 23, 43, and 33% of the accessions showed low (HDS2 = 1–2), moderate (HDS2 = 3), and high or very high (HDS2 = 4–5) damage, respectively. Combined results showed that 24 accessions had low to moderate damage (HDS = 1–3) to both herbicides that were tested for further validation. (Figure 2a). Spearman correlation between the HDS2 recorded during the preliminary screenings at both locations for metribuzin was relatively low (0.26) and highly significant ($p < .01$). Despite the significant differences for the recorded herbicide damage scores, the low correlation indicated low association between the screenings conducted in Marchouch and in Terbol as a result of environmental effects. These 24 selected accessions were screened along with three other accessions during the 2016–2017 season. The HDS1 for metribuzin treatment varied from 1 to 4, with 37% of the accessions showing very low damage (HDS1 = 1–2), 41% showing moderate damage (HDS1 = 3), and the remaining showing high or very high (HDS1 = 4–5) damage. The HDS1 score for imazethapyr treatment varied from 1 to 3, with 81% of the accessions showing very low damage (HDS1 = 1–2) and 19% showing moderate damage (HDS1 = 3). By the second scoring date, some accessions showed recovery from metribuzin and imazethapyr injuries and resulted in regrouping the accessions as follows: 70% of the accessions showed very low damage (HDS2 = 1–2) and 30% showed moderate damage by metribuzin, while all the screened accessions showed very low damage (HDS2 = 1–2) by imazethapyr 4 weeks after the treatment (Figure 2b). Figure 2 shows that the frequencies of genotypes with high HDS are higher in the preliminary screening at Marchouch 2014–2015 and Terbol 2015–2016 than in the validation screening at Marchouch 2016–

2017 and Terbol 2016–2017. The accessions showing low to moderate damage (HDS = 1–3) and no significant reduction in plant height and grain yield at Terbol and Marchouch during 2016–2017 were selected for further screening at higher dosages of herbicides at Terbol during 2017–2018. Screening against metribuzin at 250 g a.i. ha⁻¹ resulted in identification of one accession (FB2583) showing no damage (HDS1 =1). Increasing metribuzin dose to 375 and 500 g a.i. ha⁻¹ resulted in the appearance of low damage on the leaves of this accession (HDS1 = 2). The HDS2 score after 4 wk of the metribuzin treatment at 250 g a.i. ha⁻¹ showed that four accessions recovered from the herbicide injuries with no apparent damage. Score of faba bean accessions after imazethapyr at 75 g a.i. ha⁻¹ indicated no damage (HDS1 = 1) in five accessions.

These five accessions showed no (HDS1 = 1) to low (HDS1 = 2) damage even after increasing the dose to 112.5 and 150 g a.i. ha⁻¹. The HDS2 score showed no remarkable change in imazethapyr treatments at 75 and 112.5 g a.i. ha⁻¹, but in imazethapyr at 150 g a.i. ha⁻¹, six accessions showed recovery from herbicide injuries. The 2017–2018 results highlighted the regrowth capacity of the selected accessions after treatment with imazethapyr and metribuzin. These results also validated the selection of accessions in previous seasons.



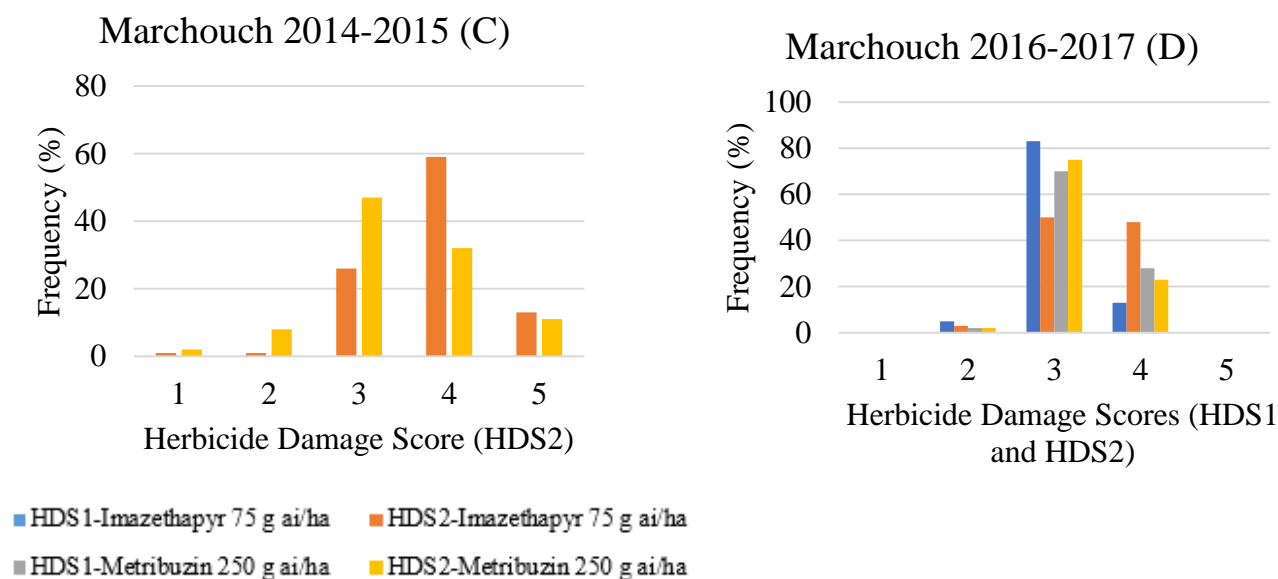


Figure 2. Distribution of faba bean accessions for herbicide damage scores (HDS1 and HDS2) under metribuzin at 250 g a.i. ha⁻¹ and imazethapyr at 75 g a.i. ha⁻¹ in preliminary (a and c) and validation screenings (b and d).

4.2. Crop phenology

The ANOVA (Table 3) showed significant differences among accessions ($p < .05$) for DFLR in all seasons at both locations except in Marchouch 2016–2017, which was exceptionally warm (Figure 1), leading to accelerated flowering. Significant differences ($p < .05$) were observed among herbicide treatments in all seasons at both locations. Significant accession \times treatment interaction was observed in Terbol 2015–2016 ($p < .001$) and Marchouch 2016–2017 ($p < .001$) for DFLR. Herbicide treatments (75 g a.i. ha⁻¹ of imazethapyr and 250 g a.i. ha⁻¹ of metribuzin) affected DFLR differently in different years and locations. Both herbicide treatments showed significant delay (Table 4) in DFLR compared with untreated plots (control) across seasons and locations. The maximum delay in flowering was observed at Terbol 2016–2017 where it varied from 114 to 138 d for metribuzin (T1) and from 116 to 136 d for imazethapyr (T2) vs. 114 to 134 d for the control. However, the earliest flowering was observed at Marchouch 2016–2017, where DFLR ranged from 46 to 50 for T1, 34 to 45 for T2, and 34 to 43 for control. These results were expected, as the season was extremely warm. During 2017–2018 season at Terbol, flowering of the tested accessions did not show any delay in T1 and T2 vs. control. These results were expected, as all accessions were selected for their tolerance to metribuzin (T1) and imazethapyr (T2) treatments. The experiment conducted at Terbol during 2017–2018 showed delay in flowering time when treated with higher

dosages of metribuzin and imazethapyr. For example, DFLR ranged from 90 to 110 d with metribuzin at 500 g a.i. ha⁻¹ (T4) as compared with 90–109 d with metribuzin at 250 g a.i. ha⁻¹. Similar observations were also obtained for imazethapyr treatments.

The ANOVA (Table 3) showed that DMAT varied significantly among the accessions ($p < .05$) and herbicide treatments ($p < .001$) across locations and seasons. The results showed no significant interaction between accessions and treatments across locations and seasons. There was a delay in maturity of accessions when treated with herbicides in comparison with the untreated ones. In Terbol 2017–2018, the delay was extended with higher dosages of herbicide treatments (T3, T4, T5, and T6) (Table 4). Days to maturity varied from 166 to 173 under T1 and from 168 to 173 under T4. Similar observations were observed for imazethapyr treatments.

4.3. Plant architecture

Plant height and BRPLT were observed to study the effect of herbicides on plant growth and development of faba bean accessions. Significant differences ($p < .001$) among accessions and herbicide treatments were observed for PLHT at both locations across the seasons. Significant interaction between accessions and treatments was also observed for PLHT ($p < .05$) in all seasons (Table 3). The mean plant height was shortened in treated plots as compared with control plots under both herbicide treatments at both locations from 2014–2015 to 2016–2017 (Table 4). Similar observations were obtained at Terbol 2017–2018 for metribuzin treatment. During 2017–2018 season at Terbol, the mean PLHT of all accessions was less when treated with higher dosages of herbicides. For example, the mean PLHT was 58 cm under T2 (imazethapyr at 75 g a.i. ha⁻¹) and 53.4 cm under T6 (imazethapyr at 150 g a.i. ha⁻¹). Similar observations were observed for metribuzin treatments (Table 4). Significant differences ($p < .001$) among accessions and herbicide treatments were observed in Terbol 2017–2018 for the BRPLT (Table 3). The mean BRPLT was higher in herbicide treated plots than in control plots (Table 4).

4.4. Yield and yield components

Grain yield per plant, SNPLT, and number of pods per plant (PNPLT), and HSW were recorded to study the effect of herbicide treatments on yield components. The ANOVA showed significant differences among accessions ($p < .001$) and treatments for grain yield in all seasons at both locations. Significant interaction between accessions and treatments was also observed for grain yield at Terbol 2015–2016 ($p < .001$) and Marchouch 2016–2017 ($p = .032$) (Table 3). The mean GYPLT was significantly lower in plots sprayed with metribuzin at 250 g a.i. ha⁻¹ (T1) or with

imazethapyr at 75 g a.i. ha⁻¹ (T2) than in control plots at both locations in all seasons except in Terbol 2017–2018 for imazethapyr (T2). Grain yield varied from 53.4 to 645 g under T1, 152.1 to 504.6 g under T2, and 101.4 to 572.7 g in control plots in Terbol 2016–2017. During 2017–2018, the average GYPLT decreased with higher dosages of metribuzin (375 and 500 g a.i. ha⁻¹) and imazethapyr (112.5 and 150 g a.i. ha⁻¹) at Terbol (Table 4).

Significant variation ($p < .05$) was observed for PNPLT and SNPLT among accessions and treatments in all years and locations. There was significant accession \times treatment interaction for both the traits at Terbol 2015–2016 ($p < .05$) (Table 3). The mean PNPLT and SNPLT were drastically reduced in all accessions when treated with herbicides in all seasons and locations except for imazethapyr treatment at Terbol 2017–2018. During 2017–2018, the mean PNPLT and SNPLT were lower when the accessions were treated with higher dosages of metribuzin and imazethapyr at Terbol. For example, mean PNPLT was 11.3 with metribuzin at 250 g a.i. ha⁻¹ and 9.9 with metribuzin at 500 g a.i. ha⁻¹ (Table 4). Similar results were observed for metribuzin treatments for SNPLT.

The effect of herbicide treatments on seed size was studied only in Terbol 2017–2018 by estimating HSW. The ANOVA showed that HSW varied significantly among accessions ($p < .001$) but not among treatments (Table 3). The mean HSW varied from 51.4 to 130.7 g among accessions (Table 4).

Table 3. Incomplete block design analysis performed for detecting significance differences for the studied traits among faba bean accessions (A), herbicide treatments (T), and A \times T interaction, expressed as P value.

		Trait ^a												
Step	Site-Season		Df	DFLR	DMAT	PLHT	RI _{HT}	PNPLT	SNPLT	GYPLT	RI _{GY}	HSW	BRPLT	
Preliminary Screening	Marchouch 2014/15	Accessions (A)	132	0.046	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.054	ND	ND
		Treatments (T)	2	ND	ND	<0.001	<0.002	0.013	0.001	<0.001	0.093	ND	ND	
		A \times T	164	ND	ND	0.057	0.932	0.279	0.019	0.998	0.481	ND	ND	
	Terbol 2015/16	Accessions (A)	133	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.05	ND	ND
		Treatments (T)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	ND	ND
		A \times T	266	<0.001	0.258	<0.001	<0.001	0.14	<0.001	<0.001	<0.001	0.051	ND	ND
Validation trial	Marchouch 2016/17	Accessions (A)	39	0.306	0.008	<0.001	0.559	ND	ND	<0.001	0.048	ND	ND	
		Treatments (T)	2	<0.001	<0.001	<0.001	0.38	ND	ND	<0.001	0.499	ND	ND	
		A \times T	78	<0.001	0.992	0.007	0.384	ND	ND	0.032	0.01	ND	ND	
	Terbol 2016/17	Accessions (A)	26	<0.001	<0.001	<0.001	0.036	<0.001	0.014	<0.001	0.637	ND	ND	

		Treatments (T)	2	<0.001	<0.001	<0.001	<0.001	<0.001	0.041	<0.001	<0.001	ND	ND
		A x T	52	0.901	0.332	0.028	0.001	0.551	0.345	0.964	0.881	ND	ND
Final validation	Terbol 2017/18	Accessions (A)	10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		Treatments (T)	6	0.022	<0.001	<0.001	<0.001	0.153	<0.001	<0.001	<0.001	0.475	<0.001
		A x T	60	0.18	0.637	0.017	0.497	0.173	0.665	0.235	1	0.95	0.146

^a DFLR days to flowering, DMAT days to maturity, PLHT plant height, RI_{HT} reduction index of plant height; PNPLT number of pods per plant, SNPLT number of seeds per plant, GYPLT grain yield per plant, RI_{GY} reduction index of grain yield per plant; HSW hundred seed weight, BRPLT number of branches per plant.

^b ND no data.

Table 4. Best linear unbiased phenotypic estimates and standard errors of different traits of faba bean accessions under different herbicide treatments at Marchouch and Terbol stations.

Step	Site, season	Treatment ^a	Trait ^b									
			DFLR	DMAT	PLHT	RI _{HT}	GYPLT	RI _{GY}	PNPLT	SNPLT	BRPLT	HSW
Preliminary screening	Marchouch, 2014–2015	T1	ND ^c	ND	53.8	24.4	13.5	34.8	11.0	21.7	ND	ND
		T2	ND	ND	52.7	25.9	11.0	46.9	10.4	18.5	ND	ND
		C	85.8	138.7	71.2	–	20.7	–	ND	ND	ND	ND
		SE	0.8	0.3	1.1	52.4	14.7	52.4	7.3	16.4	ND	ND
	Terbol, 2015–2016	T1	105.4	169.2	51.8	23.4	12.6	29.6	12.9	26.2	ND	ND
		T2	103.8	170.2	55.8	17.5	11.3	36.9	13.6	24.3	ND	ND
		C	100.4	166.7	67.6	–	17.9	–	20.9	28.3	ND	ND
		SE	0.4	0.3	0.8	13.1	0.6	44.6	2.6	2.8	ND	ND
Validation trial	Marchouch, 2016–2017	T1	47.9	106.9	48.1	29.6	13.7	49.1	ND	ND	ND	ND
		T2	40.8	103.4	52.4	23.3	16	40.5	ND	ND	ND	ND
		C	38.4	99.9	68.3	–	26.9	–	ND	ND	ND	ND
		SE	0.3	0.3	1.3	12.5	5.6	4.0	ND	ND	ND	ND
	Terbol, 2016–2017	T1	122.3	179.5	57	14.8	21.4	35.2	11.2	27.5	ND	ND
		T2	124.4	180.6	65.1	2.7	31.3	5.2	17.5	34.0	ND	ND
		C	119.8	177.8	66.9	–	33.0	–	19.0	37.6	ND	ND
		SE	0.8	0.4	0.9	9.8	6.1	5.2	0.9	3.9	ND	ND
Final validation	Terbol, 2017–2018	T1	99.1	169.1	51.1	6.8	19.4	13.4	11.3	23.7	4.4	85.8
		T2	98.9	168.2	58.0	-5.8	24.1	-7.6	12.7	29.8	5.1	86.3
		T3	99.4	169.7	48.4	11.7	16.7	25.5	10.3	21.0	3.6	85.2
		T4	101.2	1697.7	47.9	12.6	16.3	27.2	9.9	19.9	3.7	87.2
		T5	99.6	168.7	56.5	-3.1	24.8	-10.7	12.6	29.5	4.9	88.6
		T6	100.4	169.6	53.4	2.6	22.0	1.8	12.2	27.5	5.4	84.6
		C	100.7	168.2	54.8	–	22.4	–	12.0	27.0	4.4	86.1
		SE	0.7	0.5	1.2	1.6	1.8	9.3	1.1	2.3	0.3	2.9

^a T1, Metribuzin 250 g a.i. ha⁻¹; T2, Imazethapyr 75 g a.i. ha⁻¹; T3, Metribuzin 375 g a.i. ha⁻¹; T4, Metribuzin 500 g a.i. ha⁻¹; T5, Imazethapyr 112.5 g a.i. ha⁻¹; T6, Imazethapyr 150 g a.i. ha⁻¹; C, Control.

^b DFLR, days to flowering; DMAT, days to maturity; PLHT, plant height; RI_{HT}, reduction index of plant height; GYPLT, grain yield per plant; RI_{GY}, reduction index of grain yield per plant; PNPLT, number of pods per plant; SNPLT, number of seeds per plant; BRPLT, number of branches per plant; HSW, 100-seed weight.

^c ND no data.

4.5. Herbicide reduction indexes

Reduction indexes (%) were estimated for all the accessions across locations and seasons to compare the reduction in PLHT (RI_{HT}) and GYPLT (RI_{GY}) under different herbicide treatments.

Significant variation was observed for RI_{HT} among accessions and treatments in all seasons at Terbol and in 2014–2015 season at Marchouch (Table 3). There was significant accession \times treatment interaction for RI_{HT} at Marchouch 2016–2017, Terbol 2015–2016, and Terbol 2016–2017. Significant variation was observed for RI_{GY} among accessions in Marchouch 2014–2015, Terbol 2015–2016, and Terbol 2017–2018 and among treatments in all seasons at Terbol. There was significant accession \times treatment interaction for RI_{GY} at Terbol 2015–2016 (Table 3). The results showed, on an average, 34.8 and 46.9% RI_{GY} under 1 \times treatments of metribuzin and imazethapyr at Marchouch 2014–2015. Further screenings of the selected accessions during 2016–2017 showed 49.1 and 40.5% RI_{GY} under 1 \times treatments of metribuzin and imazethapyr (Table 4). Screening at Terbol 2015–2016 showed 29.6 and 36.9% RI_{GY} under 1 \times treatments of metribuzin and imazethapyr. Further screenings of the selected accessions in 2016–2017 showed 35.2 and 5.2% RI_{GY} under 1 \times treatments of metribuzin and imazethapyr (Table 4). Screenings performed in Terbol 2017–2018 for the selected accessions where additional treatments were added showed that the reduction in GYPLT increased as the herbicide dosage increases. For the metribuzin treatment, the RI_{GY} increased from negligible to 25.5% after applying 2 \times dose. On the other hand, an increase in GYPLT was observed in the case of imazethapyr treatments but this increase was reduced from 10.7 to 1.8% as the dosage applied doubled (Table 4). The RI were estimated for different accessions grouped based on their herbicide damage score (Table 5). The RI_{HT} and RI_{GY} varied significantly ($p < .05$) among the different categories of HDS under metribuzin in the preliminary screenings and validation trials conducted at Terbol and Marchouch (Table 5). The ordinal regression between RI_{HT} and RI_{GY} and the HDS was significant under metribuzin treatment where RI_{HT} and RI_{GY} increased progressively as the herbicide damage scores increased; in Terbol 2015–2016, RI_{HT} varied from 0.812% in accessions with no significant damage (HDS2 = 1) to 39.22% in those with high damage (HDS2 = 5), and RI_{GY} varied from –058% in accessions with no significant damage (HDS2 = 1) to 44.84% in those with high damage (HDS2 = 5) under metribuzin treatment. The herbicide RI_{GY} varied significantly among the accessions ($p < .05$) for imazethapyr treatment in the validation trials only and the herbicide RI_{HT} varied significantly among the accessions in the validation trial conducted at Terbol 2017–2018 only (Table 5). The ordinal regression between the HDS and RI_{GY} was significant in the validation trials and the ordinal regression between RI_{HT} and the HDS was significant in the validation trial conducted at Terbol 2017–2018 only (Table 5). Spearman correlation between HDS and the RI_{GY} and RI_{HT} are presented in Table 6. No correlation was recorded during the first site–season in Marchouch as it was conducted under rainfed conditions

with exposure to terminal drought. However, positive significant correlation between HDS and both RI_{GY} and RI_{HT} was under metribuzin treatment at Marchouch 2016–2017 but no correlation was observed for Imazethapyr treatments. Also, positive correlation between HDS and herbicide tolerance was detected in 2015–2016 at Terbol. Spearman correlation conducted between HDS recorded in the preliminary screenings at Terbol and Marchouch stations was positive, low (+0.26), and highly significant ($p < .01$).

Table 5. Ordinal regression (expressed in p value), estimate regression parameter, and best linear unbiased phenotype values of reduction index (RI , %) of plant height (RI_{HT}) and grain yield (RI_{GY}) for different levels of herbicide damage in each treatment.

Treatment	HDS2 ^a	Preliminary screenings				Validation trials			
		Terbol 2015–2016		Marchouch 2014–2015		Marchouch 2016–2017		Terbol 2016–2017	
		RI_{HT}^b	RI_{GY}^c	RI_{HT}	RI_{GY}	RI_{HT}	RI_{GY}	RI_{HT}	RI_{GY}
Metribuzin, 250 g a.i. ha ⁻¹	1	0.81	-0.58	-2.85	-33.00	–	–	46.43	13.08
	2	14.54	6.08	19.82	12.19	21.95	39.29	43.40	15.74
	3	23.59	16.51	23.68	24.27	25.49	46.94	50.46	17.98
	4	30.00	16.21	30.05	32.67	29.21	63.23	66.34	25.66
	5	39.22	44.84	24.19	41.85	–	–	98.20	48.57
	Regression (p value)	<.001	.03	.00	.01	.03	.04	<.001	<.001
	Estimate parameter ($\times 10^{-3}$)	52.73***	6.38*	37.50***	0.91*	37.90*	20.40*	79.80**	24.90**
Imazethapyr, 75 g a.i. ha ⁻¹	1	18.83	18.74	26.8	29.30	–	–	0.83	-10.48
	2	16.99	28.90	9.10	-2.60	19.34	45.57	7.61	-3.84
	3	16.10	28.85	27.46	41.62	20.58	31.73	5.24	-2.04
	4	18.71	48.53	27.48	42.76	26.45	56.48	-0.58	26.1
	5	9.74	–	26.64	17.13	–	–	–	–
	Regression (p value)	.76	.59	.87	.24	.46	.01	.01	.06
	Estimate parameter ($\times 10^{-3}$)	-2.49	1.81	-0.50	0.24	11.30	42.00*	37.30**	5.69*

^aHDS2, second herbicide damage score. ^b RI_{HT} , reduction index of plant height. ^c RI_{GY} , reduction index of grain yield per plant.

*Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level.

4.6. Selection for tolerant accessions

Values for HDS1, HDS2, RI_{HT} , mean GYPLT, and RI_{GY} of the selected accessions under different herbicide treatments are presented in Table 6. Both HDS1 and HDS2 served as a visual indication of tolerance to the herbicides that were complemented with RI_{GY} to select putative tolerant accessions. The ordinal regression analysis and Spearman correlation conducted between HDS and RI_{HT} and RI_{GY} showed that the herbicide damage scores can be used as visual indication in normal environmental conditions and under metribuzin treatment as they were significant under this

treatment only. Accessions with <15% RI_{GY} were selected as tolerant to the recommended dosage of herbicides (Table 7). Grain yield of the selected accessions did not get affected with higher dosages of imazethapyr except for IG12659 (*minor* type) and Flip 86-98FB (*major* type). However, accessions FLIP86-98FB (*major*), ILB132194 (*equina*), FB1482 (*equina*), and IG12659 (*minor*) suffered >15% reduction at higher dosages of metribuzin (Table 7).

Table 6. Spearman correlation between herbicide tolerance score (HDS) and reduction index (RI) for grain yield (GY) and plant height (PLHT) caused by metribuzin (Met) and imazethapyr (Ima) at 100% of the recommended dose.

Site, season	Trait	RI _{GYIma100}	RI _{GYMet100}	RI _{PLHTIma100}	RI _{PLHTMet100}
Marchouch, 2014–2015 (df = 132)	HDS2-Met100	-0.01	0.15	-0.07	-0.10
	HDS2 Ima100	0.04	0.14	-0.12	-0.10
Marchouch 2016–2017 (df = 39)	HDS2-Met100	0.04	0.34*	0.02	0.13
	HDS2 Ima100	0.33*	0.43**	0.01	0.32*
Terbol 2015–2016 (df = 133)	HDS2-Met100	0.05	0.40***	0.05	0.50***
	HDS2 Ima100	-0.02	0.02	0.32**	0.12
Terbol 2016–2017 (df = 26)	HDS2-Met100	0.01	0.24	-0.47*	0.35*
	HDS2 Ima100	0.41*	0.02	0.14	-0.20

*Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level.

Table 7. Herbicide damage scores (HDS1 & HDS2), plant height, grain yield per plant (GYPLT), and reduction index (RI) of grain yield (RI_{GY}) and plant height (RI_{HT}) of the selected faba bean accessions with different origin at Terbol 2017–2018 under different herbicide treatments.

Origin	Treatment ^a	Metribuzin					Imazethapyr				
		HDS1	HDS2	GYPLT	RI _{GY}	RI _{HT}	HDS1	HDS2	GYPLT	RI _{GY}	RI _{HT}
VF335 (Russia)	1×	2	1	16.8	3.1	13.3	2	1	25.5	-51.1	-4.8
	1.5×	2	2	18.3	-14.4	7.7	2	2	27.1	-59.7	-11.8
	2×	2	2	18.1	8.1	12.6	2	1	24.2	-27.2	-2.3
FB2568 (France)	1×	2	1	14.2	-36.0	-2.9	1	1	19.1	-75.2	-14.3
	1.5×	2	1	14.1	-38.2	0.2	2	1	19.7	-72.9	-7.9
	2×	2	1	13.9	-34.7	0.4	2	1	17.1	-23.9	-8.6
ILB132194 (China)	1×	3	2	24.9	-66.5	5.9	2	1	28.00	-86.6	-8.2
	1.5×	2	3	9.4	60.9	8.3	2	1	41.3	-162.7	-11.0
	2×	3	3	11.2	28.2	21.6	2	1	26.2	-66.7	2.9
FB2574 (France)	1×	2	1	19.7	4.7	8.0	1	1	22.1	-23.2	-4.1
	1.5×	2	2	25.8	-40.8	12.6	2	2	24.7	-35.7	-4.6
	2×	2	2	22.9	-25.3	14.7	2	1	17.4	2.7	7.3
FB1482 (Netherlands)	1×	2	2	13.3	-57.6	-19.1	2	1	22.7	-177.2	-37.4
	1.5×	3	3	5.2	-1.3	7.2	3	3	18.1	-112.0	-30.5
	2×	2	2	4.2	49.5	-2.5	2	2	16.4	-70.9	-29.5
IG12659 (Ethiopia) ^b	1×	2	2	20.5	36.7	7.8	2	1	29.1	10.0	-3.9
	1.5×	2	2	16.4	49.3	7.2	2	1	30.3	6.5	2.0
	2×	2	3	22.7	30.0	11.8	2	2	22.9	29.0	6.5
ILB1814 (Syria) ^b	1×	2	2	34.4	-4.1	14.9	1	1	37.5	-13.3	-4.0
	1.5×	2	2	34.2	-3.4	21.4	1	1	43.8	-32.5	10.0

	2×	2	2	35.0	-6.0	25.9	2	2	36.1	-9.1	13.0
Flip 86-98FB (Lebanon)^b	1×	2	2	24.6	30.4	10.0	1	1	30.8	12.8	-11.1
	1.5×	2	2	19.8	43.9	4.9	1	1	27.1	23.5	-16.0
	2×	2	2	25.1	29.1	9.8	2	2	29.2	17.5	-2.5
SE (accession [A])	2.6	25.5		4.4							
SE (treatment [T])	1.8	13.9		2.6							
p value (A)	<.001	<.001		<.001							
p value (T)	<.001	.005		<.001							
p value (A × T)	.235	.658		.324							

^a1×, 100% of the recommended dose; 1.5×, 150% of the recommended dose; 2×, 200% of the recommended dose.

^bAccessions selected at both Terbol and Marchouch locations in 2016–2017 season.

5. Discussion

Field experiments with faba bean germplasm confirmed that postemergence application of metribuzin and imazethapyr can cause severe damage by affecting phenology, vegetative growth, grain yield, and yield components. This confirmed previous reports on faba bean (García-Torres et al., 1991; Maalouf et al., 2016a; Sharma et al., 2018) and other legume crops that herbicide application caused severe damage to the crops (Gaur et al., 2013; Jefferies et al., 2016).

5.1. Response to herbicide treatments

The HDS observations were variable across the locations and seasons. The variation observed in the recovery of some faba bean accessions and increased damage of other accessions after 1 mo of spray was expected, as the screened accessions were very diverse with no previous history of selection for herbicide tolerance. However, when selected accessions were re-evaluated in normal environmental conditions, most accessions recovered from the herbicide injuries. Our results are similar to the one observed by Sharma et al. (2018) in lentil cultivars treated with metribuzin. The recovery mechanism of plants from the herbicide treatments could be metabolism based, as the herbicides, and might be metabolized into inactive compounds, allowing the acetolactate synthase (ALS) enzyme to regain its activity in the imazethapyr treated plants (Teclé et al., 1993). This phenomenon of detoxification of imidazoline was also observed in soybean (Teclé et al., 1993). On the other hand, when selected accessions were re-evaluated under drought-like conditions, only few accessions could recover from the herbicide damage as it would be expected from tolerant genotypes in normal wet years, as reported in chickpea (Taran et al., 2010), where plants recovered from the herbicide damage as the season progressed under favorable weather conditions. Drought-like conditions led to the significant bias in HDS as water stress compounded the symptoms of

herbicide damage. The difference in the behavior of accessions evaluated under different environmental conditions explains the low correlation observed between the HDS recorded in two different sites.

Our study revealed also that the injuries caused by herbicide treatments increased with the increase in the concentration of metribuzin and imazethapyr. Similar observations were recorded by Goud et al. (2013) in chickpea treated with imazethapyr.

5.2. Effects of herbicide on crop phenology

A delay in flowering and maturity of faba bean accessions was observed with metribuzin and imazethapyr application at postemergence stage in all sites and years. This is in agreement with the earlier reports in chickpea and lentil (Gupta et al., 2017; Jefferies et al., 2016; Sajja et al., 2015; Taran et al., 2013). The delay in flowering time might be due to temporary inhibition of growth of treated plants, which also caused delay in maturity. Gaston et al. (2002) suggested that the inhibited growth in pea after imazethapyr treatment was due to the impairment of ALS activity that led to the death of meristematic cells. Metribuzin treatment also inhibited the growth of narrow-leaf lupin and chickpea plants by inhibiting the photosynthesis activity (Gaur et al., 2013; Pan et al., 2012).

5.3. Effect of herbicide on plant architecture

Reduction in PLHT of faba bean accessions was observed after spraying with metribuzin and imazethapyr across all locations and years. Field experiments conducted by Taran et al. (2010) and Sajja et al. (2015) also confirmed that post-emergence application of imazethapyr reduced PLHT in chickpea. Similar results were observed by Sharma et al. (2016, 2018) in lentil when treated with metribuzin and imazethapyr. The reduction in PLHT of imazethapyr- and metribuzin-treated plants might be due to growth inhibition effect of herbicides (Aboali et al., 2015; Gaston et al., 2002) and the observed variation in the RI_{HT} between tolerant and susceptible accessions might be due to differential metabolic degradation rate in the case of imazethapyr treatment (Sharma et al., 2018) and to differential disruption of electron transfer in the case of metribuzin treatment. Unlike PLHT, we observed an increase in the BRPLT of herbicide treated plants. This agrees with increased BRPLT reported in faba bean (El Mahi, 1991), lentil (Wall, 1996), and chickpea (Sajja et al., 2015) after postemergence imazethapyr treatment. The increased BRPLT could be caused by the plant regrowth that occurs at the lateral meristem in the dicots, which resulted in the plant developing new branches.

5.4. Effect of herbicide on yield components

The postemergence application of imazethapyr and metribuzin caused significant reduction in GYPLT and PNPLT. Similar results were observed in lentil (Friesen et al., 1986; Sharma et al., 2016, 2018) and chickpea (Taran et al., 2010) cultivars treated with metribuzin and imazethapyr at postemergence stage. Narrow leaves that were observed after imazethapyr spray reduced the leaf area index (Maalouf et al., 2016a) and therefore affected photosynthetic activity, which led to a poor canopy coverage that ultimately reduced GYPLT. The same holds true for metribuzin application as it is known that metribuzin inhibits photosynthesis. In this study, HSW was not affected by the herbicide treatments. However, contrary to the results of the present study, significant reduction in seed size was observed by Sharma et al. (2018) in lentil where herbicide-treated plots of all accessions showed significant decrease in the size and volume of seeds.

5.5. Selection for herbicide tolerance

Herbicide damage scores are relatively easy observations, allowing preliminary ranking of accessions. However, in the case of imazethapyr treatment, these scores did not always correlate with RI_{GY} and RI_{HT} , showing that apparently tolerant accessions showing low damage might still be suffering a significant yield and height and therefore not be as tolerant as identified by HDS score only and that some accessions grouped as tolerant did not suffer a significant yield and height reduction as the crop cycle delay caused by the imazethapyr treatment allowed their recovery under normal environmental conditions. Both RI_{GY} and RI_{HT} are considered more reliable to assess herbicide tolerance and should at least complement the preliminary HDS observations. Therefore, in the present study, the selection for metribuzin and imazethapyr tolerance was based mainly on the reduction of the PLHT and GYPLT as the visual observation was not enough for having a fair grouping of the evaluated genotypes. Our selection method is similar to the one conducted by Burgos et al. (2007), which selected cowpea breeding lines tolerant to herbicide treatment based on their yield reduction. However, Gaur et al. (2013) and Sharma et al. (2018) selected several chickpea and lentils genotypes tolerant to metribuzin and imazethapyr based on the visual scoring as they found that the reduction of the yield was directly correlated to the level of tolerance or sensitivity of the genotypes to herbicide treatment.

6. Conclusions

The present study shows that postemergence application of imazethapyr and metribuzin causes delay in flowering and maturity and a reduction in plant height, yield components, and grain yield.

The results showed enough natural genetic variability in faba bean germplasm for tolerance to metribuzin and imazethapyr herbicides. Visual observation of HDS is handy and rapid criterion for screening large number of germplasm accessions and selecting putative germplasm for further testing of their reaction. Environmental conditions affected recovery of the treated accessions, especially that they mature later than the untreated ones; we suggest assessing HDS 2 mo after the herbicide spray when pods would already be formed, and recovery would be clearer as the season progress. The RI_{GY} appeared to be the most relevant criteria for assessing the herbicide tolerance. By using HDS solely as selection criteria, we might end up retaining accessions with significant yield reduction after the herbicide treatment even when showing little visually noticeable damage. Therefore, the use of yield RI is more appropriate criterion for confirming actual tolerance of selected accessions. Herbicide tolerant faba bean accessions identified in this study can be used in crossing programs to transfer herbicide tolerance into cultivars adapted to different agroecological zones and also to conduct genetic studies to dissect and characterize its components. A genome-wide association study could be conducted to identify markers associated with herbicide tolerance to establish marker–trait association for marker assisted selection of herbicide tolerant breeding lines at an early generation.

7. References

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CHAPTER III

Adaptability and Stability of Faba Bean (*Vicia faba* L.) Accessions under Diverse Environments and Herbicide Treatments

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1. Abstract

The adaptability and stability of 37 faba bean (*Vicia faba* L.) accessions with different levels of tolerance to metribuzin or imazethapyr was assessed across 12 season–location–herbicide experiments. Significant Genotype x environment (GE) interaction was found for the days to flowering (DFLR), plant height (PLHT) and grain yield (GY). Performance and stability of the accessions regarding PLHT and GY were assessed using four different stability parameters: cultivar superiority, static stability, Wricke’s eco-valence and Finlay and Wilkinson’s regression model. The stability parameters ranked these genotypes differently suggesting that PLHT and GY stability should be assessed not only on a single or a few stability parameters but on a combination of them. GGE biplot analysis indicated that the environments representing metribuzin treatment at Marchouch 2014–2015 and the non-treated treatment at Terbol 2018–2019 are the ideal environments for evaluating faba bean genotypes. GGE biplots showed herbicide tolerant accession IG12983 with simultaneous average PLHT, GY and stability across the environments. The performance of other tolerant accessions, namely IG13945, IG13906, IG106453, FB2648, and FB1216 was less stable but superior under specific mega environments. Therefore, utilizing these accessions in faba bean breeding programs would help broaden the adaptability to diverse locations–season–herbicide treatments.

2. Keywords

Faba bean; herbicide tolerance; $G \times E$ interaction; stability parameters; GGE biplot.

3. Introduction

Faba bean (*Vicia faba* L.) was domesticated 10,000 years Before Christ (BC) in the Near East where archeological findings of domestic [1] and wild specimens were discovered [2]. Today, faba bean is considered the fourth most important cool season food legume after chickpea, lentils, and peas as it is grown on 2.57 million ha area with a total production of 5.4 million tons in 2019 [3]. This crop plays critical role in supporting nutritional and food security and enhancing soil structure in many countries, including China, Egypt, Ethiopia, United Kingdom, Australia, France, Sudan, and Morocco [3]. Faba bean production has increased 2% annually over the past three decades while global area remains stagnant.

Faba bean is affected by several biotic and abiotic stresses including parasitic and non-parasitic weeds. Among annual weeds, broadleaved and grass species like *Anthemis arvensis* L., *Chenopodium album* L., *Convolvulus arvensis* L., *Sinapis arvensis* L. and *Avena sterilis* L. compete with faba bean crop [4,5]. Parasitic weeds like *Orobanche crenata* L. and *Cuscuta sativa* L. also severely affect faba bean in many production areas [6,7,8]. An integrated weed management that employs a variety of chemical and non-chemical methods is an effective way of minimizing the losses caused by weeds. The development of resistant cultivars to multiple herbicides with different modes of action [9,10,11] is still the most economical and environmentally friendly control strategy to reduce the cost of the weed control practices, and avoid herbicides injury to the crop and herbicide resistance of some weeds [9,12,13]. Research conducted at ICARDA [9,14] resulted in the identification of accessions that tolerate metribuzin and/or imazethapyr herbicides. However, demonstrating the adaptability of these accessions to a wide range of environments can increase their economic value as climate change is expected to reduce the production of faba bean in many regions. Therefore, there is a need to study the yield stability of these accessions under different environmental conditions.

Grain yield is a very complex trait which is strongly influenced by genotype (G), environment (E) and genotype x environment (GE) interaction [15,16]. GE interaction is of major importance for breeders, given that it reduces the association between phenotypic and genotypic values across environments [15,17]. It also affects the identification of relevant test environments, the allocation of resources within a breeding program and the choice of germplasm and breeding

strategy [18]. GE interaction is a challenge in the case of legume breeding as previous studies have suggested a high proportion of variance due to environment (E) and GE interaction on the expression of grain yield in pulse crops including faba bean [19,20].

Environmental variation has a major effect on the variation of yield (up to 80% or higher) [21] in developed pure lines with narrow genetic base, but genotypes and GE interaction are more relevant for germplasm evaluation and selection and they must be considered simultaneously when selecting a genotype; in other words, an ideal genotype should have both high mean yield performance and high stability across environments [22,23].

The major objective of this research was to assess yield stability of selected faba bean accessions for herbicide tolerance under different environments with combined effect of herbicide treatment, location, and season. The second objective was to identify mega-environments and the best environments to screen faba bean for herbicide tolerance.

4. Materials and Methods

4.1. Materials

Thirty-seven faba bean accessions with different level of tolerance to one or both herbicides, metribuzin and imazethapyr, were evaluated in this study. Thirty-six accessions were pure lines derived from single plant selections in three consecutive seasons which represent landraces from 21 countries and one cultivar Elisar (Flip 86-98FB) released in Lebanon was used as check (Table S1). Among them, 14 were tolerant or moderately tolerant to both the herbicides @ 250 g ai/ha metribuzin and 75 g ai/ha imazethapyr, six were tolerant to 250 g ai/ha metribuzin but sensitive to imazethapyr, and 16 were tolerant to 75 g ai/ha imazethapyr but sensitive to metribuzin [9, ICARDA, unpublished data].

4.2 Experiments

A total of 12 experiments combining locations, seasons and herbicide treatments were conducted at Marchouch in Morocco (33.56° N, 6.69° W, 255 m) during the main cropping seasons of 2014/2015, and Terbol in the Bekaa Valley of Lebanon (35.98° N, 33.88° E, 890 m) during 2015/2016, 2016/2017 and 2018/2019 as detailed in Table 7. Each combination of herbicide treatment, location and season was considered as an independent environment. The experiments were conducted in an incomplete block design with two replicates. The plot size was 2 rows with one-meter length with rows spaced at 45 cm and plants spaced at 20 cm within the row. Terbol station is characterized by a cool high rainfall winter and a moderate wet spring. The rainfall

recorded during 2015/2016 and 2017/2018 winter cropping seasons were 343 mm and 810.2, respectively. A supplemental irrigation of 30 mm was provided during 2015/2016 to compensate the dry spell periods. Spring 2017/2018 was warmer than normal spring seasons (Table 1). The soil in Terbol station is deep and rich clay loam. Marchouch station is characterized by semi-arid environment, low rainfall and moderate temperature during winter and spring seasons. The annual rainfall recorded during 2014/2015 and 2016/2017 cropping seasons were lower than the mean annual rainfall (396 mm) and the spring season of 2016/2017 was relatively warmer than normal spring seasons. The soil at Marchouch is Vertisol mostly silty clay (Table 1).

The experiments were supplied with 250 kg/ha of granulated NPK (15:15:15) during land preparation. The experiments were sown in late November at Terbol and mid-December at Marchouch and harvested by the end of May at both locations. Necessary phytosanitary and agronomic management practices were applied to ensure a good crop stand: lambda-cyhalothrin @ 40 g ai/ha was sprayed to control sitona weevil (*Sitona lineatus* L.), imidacloprid @ 160 g ai/ha was sprayed to control aphids and azoxystrobin @ 72.8 g ai/ha and difenoconazole @ 45.6 g ai/ha were sprayed alternatively to control foliar diseases. To control weeds in all environments, we applied 1200 g ai/ha of pendimethalin as preemergence treatment in addition to post-emergence herbicides. For post-emergence herbicide treatments, two herbicides, namely Metribuzin @250 g ai/ha and Imazethapyr @75 g ai/ha were sprayed uniformly at the stage of inflorescence emergence-BBCH stage 50 [82,83] in addition to a control untreated treatment where hand weeding was applied to ensure an unbiased evaluation of the performance and stability of the faba bean accessions as they were evaluated under the same conditions except for the herbicide treatment

Table 1. Details of different environments where the faba bean accessions were tested.

Environment Symbol	Environment (Site-Season-Treatment Details)	Rainfall (mm)	Supplemental Irrigation (mm)	Air Temperature (°C)		
				Average	Average Min	Average Max
A	Marchouch-2014/2015 treated by metribuzin 250 g ai/ha	291.4	0	13.12	5.61	23.64
B	Marchouch-2014/2015 treated		0			

	by imazethapyr 75 g ai/ha					
C	Marchouch- 2014/2015 with no herbicide treatment		0			
D	Marchouch- 2016/2017 treated by metribuzin 250 g ai/ha	211	0	14.05	-2.4	42.99
E	Marchouch- 2016/2017 treated by imazethapyr 75 g ai/ha		0			
F	Marchouch- 2016/2017 with no herbicide treatment		0			
G	Terbol- 2015/2016 treated by metribuzin 250 g ai/ha	343	30	11.5	-0.44	24.62
H	Terbol- 2015/2016 treated by imazethapyr 75 g ai/ha		30			
I	Terbol- 2015/2016 with no herbicide treatment		30			
J	Terbol- 2018/2019 treated metribuzin 250 g ai/ha	810.2	0	11.7	-0.28	32.3

K	Terbol- 2018/2019 with Imazethapyr 75 g ai/ha	0
L	Terbol- 2018/2019 with no herbicide treatment	0

Min-Minimal temperature during cropping season; Max-Maximal temperature during cropping season.

4.3 Recorded Traits

The following traits were measured based on the faba bean ontology described by Maalouf [84]: days to flowering (DFLR) and days to maturity (DMAT) were recorded on plot basis while grain yield per plant (GY) and plant height (PLHT) were recorded on three random plants from each plot and averaged.

4.4 Statistical Analysis

The spatial statistical model was applied for variance analysis using the Automatic Spatial Analysis of incomplete block design modules of GenStat 19 edition [85]. Variation among accessions and treatments was assessed in terms of *p*-values using the Wald statistic, and the best unbiased phenotypic estimates of accessions (A) were estimated with standard error using best linear unbiased prediction values (BLUP) using GenStat software. Narrow-sense heritability for each environment (h^2) was estimated for the PLHT and GY using the method of residual maximum likelihood (REML) and combined narrow sense heritability (h^2) was estimated for the traits based on the combined analysis using REML model and Best unbiased estimated values of Genstat 2019. In our study the environment is defined as the combination of year–locations and herbicide treatments either metribuzin or imazethapyr. The following four stability parameters were assessed using GenStat software by comparing different treatments and environments: cultivar superiority index [61], which refers to the ability of the accession to perform above the mean in different environments, static stability coefficient; [86] which refers to the consistency of accession’s performance across different environments; Wricke’s ecovalence [24], which refers to the contribution of the accession to GE interaction; and the index of Finlay and Wilkinson [25], which

refers to the response of an accession to different environments by fitting a regression of the environment means for each accession on the average environmental means.

The GGE biplot is an ideal tool for the analysis of data from multi-environment trials (MET); it considers both G and GE interaction effects and graphically displays GE interaction in a two-way table [87]. GGE biplot allows visual examination of the relationships among the test environments, the performance and stability of the genotypes and the mega-environment analysis to recommend specific genotypes for specific mega-environments [16,20,26].

GGE biplot analyses of tested accessions were conducted using the BLUPs obtained under diverse herbicide treatments at two locations: Terbol which is characterized by high rainfall, and Marchouch by low rainfall. The environments with low narrow sense heritability for both GY and PLHT were excluded from the GGE biplot analysis as most of the variations are related to the environmental conditions. The relationship between the environments was visualized by drawing a vector that connected each environment to the biplot origin:

- The correlation between two environments was approximated based on the angle between two vectors [26,88]; the smallest the angle between two vectors, the highest is the correlation between the two environments.
- The discriminating ability of the test environments was evaluated based on the length of the vector of each environment, the longer the environment vector, the more the discriminating ability of the environment.

The best genotypes for each environment and the possibility of existence of mega environments were identified using the “who-win-where” visualization [29,87]; the polygon view of a biplot (convex hull) is the best way to visualize the interaction between the genotypes and the environments [16]; each polygon was formed by connecting the genotypes that are farthest from the biplot origin so that all other genotypes are inside the polygon [20]. The perpendicular lines to the sides of the polygon divide the biplot into sectors. Each sector has a vertex genotype. The vertex genotype is the one having the longest vector and is considered the winning genotype. The mega-environment was identified as the group of environments that share the same winning genotypes following Yan and Rajcan [27].

A genotype is considered superior when it has both high mean performance and high stability across the test environments. The mean yield performance and stability of genotypes were evaluated by an average environment coordination (AEC) method [27,29]:

- The mean performance of the genotype was graphically evaluated based on the line perpendicular to the average tester axis (ATA) that passes through the origin and separates entries with below-average means from those with above-average means; the genotypes located on the right side of this line are taller or have more yield than the ones located on the left side.
- The stability of the accessions was graphically represented by the projection from the genotype to the ATA; the longer the projection the greater is the GE interaction and therefore the lower the stability of the genotype across environments.

5 Results

5.1 Phenological traits

Combined analysis of variance showed significant differences among the 37 accessions across the 12 environments for days to flowering (DFLR) and days to maturity (DMAT) reflecting the presence of genotypic variability for both traits. In addition, the interaction between Genotype x Herbicide treatment was highly significant (Table 2) indicating that the studied genotypes behaved differently under different herbicide treatments for DFLR and DMAT in the different environments. Very high values of narrow sense heritability (0.97 and 0.99) for both DFLR and DMAT were estimated under different herbicide treatments and across different locations-seasons (Table 2).

Table 2. Combined analysis performed for detecting Wald statistics and differences among faba bean genotypes, treatments, and genotypes x treatment interaction for phenological and agronomic traits across environments.

	df	DFLR	DMAT	PLHT	GY
Genotype × Environment	396	949.1 ***	206	728.8 ***	800.4 ***
Herbicide treatments (T)	2	2.52	4.8	36.56 ***	33.5 ***
Genotypes (G)	36	1859.3 ***	199.2 ***	278.1 ***	268.1 ***
G × T	72	156.5 ***	97.1 *	70.7	125.5 ***
h^2	-	0.97	0.99	0.60	0.40

df: degree of freedom, DFLR: days to flowering, DMAT: days to maturity, PLHT: plant height, GY: grain yield,

* $p < 0.05$, *** $p < 0.001$, h^2 narrow sense heritability.

The analysis of variance conducted for each environment showed significant differences ($p < 0.001$) among accessions in all environments for DFLR except in Marchouch 2016/2017-

imazethapyr (environment E), where the season was dry and warm. The flowering time was earlier in the environments C, F, I and L where no herbicide treatment was applied at both locations and in different cropping seasons. Means and ranges of DFLR are presented in Table 2. This varied among accessions from 39 days at Marchouch 2016/2017 without herbicide treatments (environment F) to 53 days after sowing (DAS) at Marchouch 2016/2017-imazethapyr treatment (environment E) and the widest range was observed in Terbol 2015/2016-imazethapyr treatment (environment H) where it varied from 93 to 131 DAS.

The analysis of variance revealed significant differences ($p < 0.001$) among accessions in all environments for days to maturity (DMAT) except in environments E, H and K where they were treated with 75 g ai/ha of imazethapyr in Marchouch 2016/2017 and Terbol 2016/2017 and 2018/2019 seasons.

The maturity time was delayed in trials treated by both metribuzin and imazethapyr herbicides over season–locations than those with no herbicide application (Table 3). The narrowest range of maturity time was observed in Terbol-2015/2016 with no herbicide treatment (environment I) where it varied between 165 and 171 DAS, and the widest range was observed in Marchouch 2014/2015 with no herbicide treatment (environment C) where it varied from 131 to 147 DAS (Table 3).

Table 3. Means \pm Standard error (SE) and ranges for different traits in the different environments.

Environment	Environment Details	Means	DFLR	DMAT	PLHT (cm)	GY (Kg/ha)
A	Marchouch 2014/2015 treated by metribuzin 250 g ai/ha	Range	ND	ND	28–78	440–5995
		Mean \pm SE	ND	ND	55.8 \pm 1.5	2344 \pm 620
B	Marchouch 2014/2015 treated by imazethapyr 75 g ai/ha	Range	ND	ND	37–82	220–6380
		Mean \pm SE	ND	ND	55.1 \pm 8.9	1450 \pm 1158
C	Marchouch 2014/2015 with no herbicide treatment	Range	78–97	131–147	50–112	333–7333
		Mean \pm SE	84.9 \pm 3.81	137.9 \pm 3.6	72.5 \pm 7.5	3545 \pm 1360
D	Marchouch-2016/2017 treated by metribuzin 250 g ai/ha	Range	37–47	100–109	32–73	363–3091

		Mean ± SE	41.1 ± 1.6	103.8 ± 1.9	52.1 ± 8.2	1857 ± 392.2
E	Marchouch-2016/2017 treated by imazethapyr 75 g ai/ha	Range	45–53	101–111	28–75	330–2486
		Mean ± SE	47.9 ± 1.6	107 ± 2.26	48.2 ± 9.9	1385 ± 392
F	Marchouch-2016/2017 with no herbicide treatment	Range	34–44	96–106	41–82	1089–3729
		Mean ± SE	39.3 ± 2.0	99.91 ± 1.8	65.4 ± 5.7	2470 ± 499.5
G	Terbol-2015/2016 treated by metribuzin 250 g ai/ha	Range	93–130	165–173	18–77	0–4190
		Mean ± SE	103.6 ± 4.4	168.8 ± 2.3	51.8 ± 8.2	1720 ± 665
H	Terbol-2015/2016 treated by imazethapyr 75 g ai/ha	Range	93–131	165–173	33.0–76.7	57–3689
		Mean ± SE	102.8 ± 3.0	170.1 ± 2.75	57.1 ± 7.3	1439 ± 739.8
I	Terbol-2015/16 with no herbicide treatment	Range	93–122	165–171	39.3–93	352–4184
		Mean ± SE	98.8 ± 1.6	166.1 ± 1.97	68.5 ± 7.3	2313 ± 474
J	Terbol-2018/2019 treated metribuzin 250 g ai/ha	Range	99–130	175–183	49–105	321.2–4782
		Mean ± SE	107.1 ± 2.1	178.7 ± 1.53	79.7 ± 7.98	2423 ± 745.3
K	Terbol-2018/2019 with Imazethapyr 75 g ai/ha	Range	99–130	175–185	48–103	781–5369
		Mean ± SE	106.8 ± 2.3	175 ± 1.33	72.3 ± 8.3	2757 ± 679
L	Terbol-2018/2019 with no herbicide treatment	Range	93–130	175–183	52–113	912–7788
		Mean ± SE	106 ± 2.1	176.8 ± 0.8	83.5 ± 7.6	3424 ± 1173

SE. standard error, DFLR days to 50% flowering after sowing, DMAT days to 80% maturity, PLHT plant height, GY yield, ND. no data.

5.2 Plant Height

Combined analysis of variance showed that plant height varied significantly among genotypes and herbicide treatments, but no significant GE and Genotype × Herbicide Treatment interactions were observed across environments (Table 2, Figure 1). However, significant differences among

genotypes for plant height were detected in all environments except in Marchouch during 2016/17- imazethapyr treatment (environment E) where severe terminal drought occurred. Narrow sense heritability of plant height was relatively high (0.60) indicating replicability of the traits among accessions in different herbicide treatments and across different locations-seasons (Table 1). h^2_{PLHT} varied between 0.01 in Terbol 2015/2016-no herbicide treatment (environment I) and Terbol 2018/2019-metribuzin treatment (environment J) and 0.95 in Marchouch 2014/2015-metribuzin treatment (environment A) (Table 4). However, significant GE interaction was also observed for plant height indicating that the genotypes responded differently in different seasons and locations (Table 2).

All tested accessions had lower plant height under metribuzin and imazethapyr than under no herbicide application (Table 3). Average plant height varied from 18 cm in Terbol-2015/2016–metribuzin treatment (environment G) and 112 cm in Terbol-2018/2019-no herbicide treatment (environment L). Figure 1 showed that non-treated plants tended to have the highest height, followed by the plants treated with metribuzin and then by those treated with imazethapyr. The plant height of accession IG104039 classified previously as tolerant to both herbicides did not differ significantly under metribuzin or imazethapyr treatments across all environments, and the plant height of accession VF513 classified previously as tolerant to metribuzin did not differ significantly under metribuzin treatment across environments (Figure 1).

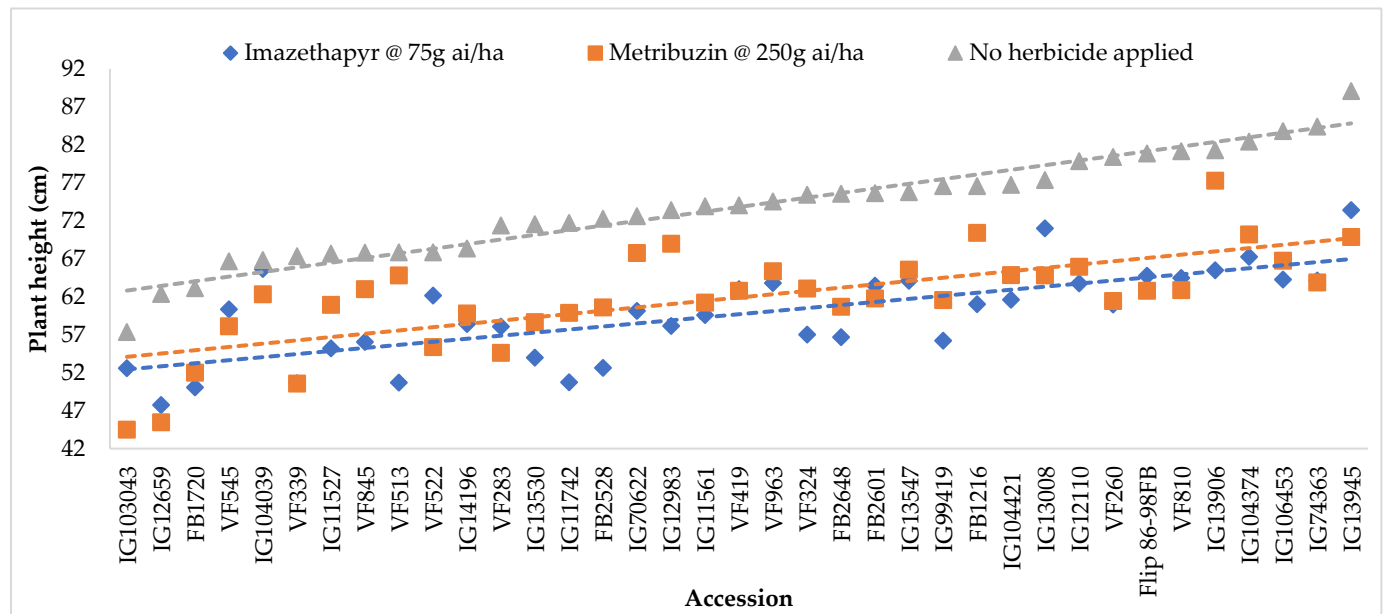


Figure 1. Mean plant height (cm) of studied accessions of faba bean across seasons–locations under imazethapyr, metribuzin and no herbicide application treatments (SE = 5.149).

Table 4. Wald statistics performed estimates for detecting differences among faba bean genotypes and narrow sense heritability of plant height and grain yield across environments.

Environment	Environment Characteristics	df	PLHT	h^2 _PLHT	GY	h^2 _GY
A	Marchouch 2014/2015 treated by metribuzin 250 g ai/ha	36	3755.1 ***	0.95	385.6 ***	0.50
B	Marchouch 2014/2015 treated by imazethapyr 75 g ai/ha	36	99.1 ***	0.50	107.1 ***	0.50
C	Marchouch 2014/2015 with no herbicide treatment	36	203.4 ***	0.50	62.7 **	0.50
D	Marchouch-2016/2017 treated by metribuzin 250 g ai/ha	36	64.9 **	0.03	69.8 *	0.00
E	Marchouch-2016/2017 treated by imazethapyr 75 g ai/ha	36	46.3	0.05	82.1 *	0.00
F	Marchouch-2016/2017 with no herbicide treatment	36	82.1 *	0.32	83.5 *	0.00
G	Terbol-2015/2016 treated by metribuzin 250 g ai/ha	36	149.7 ***	0.18	170.5 ***	0.01
H	Terbol-2015/2016 treated by imazethapyr 75 g ai/ha	36	95.0 ***	0.14	89.7 ***	0.06
I	Terbol-2015/2016 with no herbicide treatment	36	135.0 ***	0.01	352.5 ***	0.12
J	Terbol-2018/2019 treated metribuzin 250 g ai/ha	36	74.9 *	0.01	139.2 ***	0.00
K	Terbol-2018/2019 with Imazethapyr 75 g ai/ha	36	97.9 ***	0.01	130.7 ***	0.24
L	Terbol-2018/2019 with no herbicide treatment	36	160.1 ***	0.02	74.4 *	0.00

Df. degree of freedom, PLHT plant height, h^2 _PLHT narrow sense heritability of plant height, GY grain yield, h^2 _GY narrow sense heritability of grain yield, GE Genotype \times Environment interaction, ND. no data, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

As significant GE interactions were detected for plant height, the stability parameters were assessed to determine the specific response of the tested accessions using the four stability parameters, namely cultivar superiority, static stability, Wricke's eco-valence [24] and Finlay and Wilkinson [25] stability parameter.

The rankings of the accessions based on the plant height stability are presented in Table 5. Considering the cultivar superiority and the ability of the genotypes to have a mean plant height

above average across environments, IG13945 had the best plant height performance across environments. As for the ability of the genotypes to maintain a stable performance across environments, FB2601 was considered the most stable based on the static stability parameter, VF845 based on Wricke's eco-valence, as it had the lowest eco-valence value, and IG12659 based on Finlay and Wilkinson stability parameter, as it received the lowest values for these parameters.

Table 5. *Cultivar superiority index, static stability, Wricke's eco-valence and Finlay and Wilkinson values for plant height of faba bean accessions evaluated in 12 different environments. Values marked in bold belong to the 10 most stable accessions, while values presented between brackets reflect their ranking.*

Accession	Accession Number	Cultivar Superiority	Static Stability	Wricke's Eco-valence	Finlay-Wilkinson
IG11561	1	331.9(23)	189.4(19)	519.8(14)	0.9074(16)
IG12110	2	170.6(4)	189.4(18)	515.7(13)	0.9941(18)
VF283	3	351.7(26)	218.2(23)	750.1(24)	1.1284(27)
IG13906	4	199.2(6)	155.3(12)	764.4(25)	0.8296(12)
IG74363	5	157.7(3)	289.9(31)	834.9(27)	1.2719(32)
IG13530	6	342.3(25)	207.5(21)	201.2(2)	1.2292(30)
IG13547	7	216.6(10)	238.5(28)	620.5(18)	1.0971(24)
VF513	8	399.6(28)	143(10)	664.3(20)	0.9026(15)
VF522	9	429.5(31)	206.1(20)	912.3(30)	1.0141(19)
IG11742	10	381.7(27)	231.9(26)	498.4(11)	1.142(28)
IG12659	11	646.1(36)	120.5(6)	1312.2(33)	0.5057(1)
VF419	12	266.6(17)	161.4(14)	360.3(5)	0.9258(17)
IG104039	13	401.7(29)	563.2(36)	2596.2(37)	1.7049(37)
FB2648	14	327.4(21)	270.4(30)	557.2(16)	1.2729(33)
FB2528	15	402.1(30)	157.6(13)	514.3(12)	0.8565(14)
FB2601	16	212.1(8)	104.1(1)	540.6(15)	0.6932(6)
IG104374	17	155.1(2)	207.8(22)	368.3(6)	1.1143(25)
IG104421	18	216.4(9)	174.5(16)	271.8(3)	1.0751(22)
IG106453	19	177(5)	162.2(15)	641.8(19)	0.8476(13)
Flip 86–98FB	20	199.6(7)	129.9(8)	298.8(4)	1.0777(23)
IFB1216	21	220.4(11)	227.5(25)	672.2(21)	1.1187(26)
IG11527	22	328.2(22)	118.3(5)	742.4(23)	0.7(7)
VF845	23	339.8(24)	139(9)	181.3(1)	1.0289(20)

IG99419	24	326.1(20)	178.8(17)	831.6(26)	0.7987(11)
VF324	25	284.5(19)	225.1(24)	905.8(29)	1.0561(21)
VF339	26	557.5(34)	125.1(7)	471.9(9)	0.6844(5)
VF963	27	227.3(13)	-	477.7(10)	0.7321(8)
IG13945	28	84.7(1)	390.2(35)	2204(36)	1.249(31)
IG14196	29	447.2(33)	357.2(33)	1382.4(34)	1.3134(34)
FB1720	30	565.3(35)	107.3(2)	680.9(22)	0.641(3)
IG13008	31	263.8(16)	379.8(34)	1229.9(31)	1.5122(36)
IG103043	32	717.7(37)	263.8(29)	2035.4(35)	0.7726(10)
IG70622	33	272.5(18)	114(4)	437.8(8)	0.7637(9)
VF545	34	441.5(32)	143.4(11)	1299.7(32)	0.5994(2)
IG12983	35	263.7(15)	112.7(3)	599.3(17)	0.6791(4)
VF810	36	222.1(12)	233.7(27)	413.1(7)	1.1556(29)
VF260	37	231.4(14)	351.6(32)	839.4(28)	1.4862(35)

Among the genotypes that had plant height above average and performed well in all environments, only four FB2601, IG104374, IG104421 and Flip86-98FB had small fluctuation across environments and were identified as stable by two of the four stability parameters. Among these genotypes classified earlier as moderately tolerant/tolerant to both herbicides, IG104374, IG104421 and Flip86-98FB were identified as stable by Wricke's eco-valence and FB2601 and Flip 86-98FB by the static stability parameter. Wricke's eco-valence followed by the static stability parameter was effective in simultaneously selecting stable genotypes with high plant height unlike the Finlay and Wilkinson's stability parameters which, in this study, identified mostly genotypes with low plant height as being the most stable.

The correlations among the stability parameters are shown in Table 6 The correlation coefficient varied between -0.4 and 0.9 indicating an inconsistency in the classification between the parameters. Significant negative correlation (-0.4) was observed between Cultivar superiority and Finley and Wilkinson's parameter and highly significant and positive correlation was observed between Finley and Wilkinson's parameter and Static stability (0.9) and between Wricke's eco-valence and Static stability (0.7). However, even with strong correlation between methods, genotype ranking can be different. FB2601, Flip86-98 and IG70622 had the most stable plant height as they ranked among the 10 most stable accessions by different stability parameters.

Table 6. Correlation coefficients between the stability parameters of the 37 faba bean accessions tested across 12 environments.

Trait	Method	Cultivar Superiority	Finlay and Wilkinson	Static Stability
PLHT	Finlay and Wilkinson	-0.4 *	-	-
	Static stability	-0.1	0.9 ***	-
	Wricke's eco-valence	0.3	0.3	0.7 ***
GY	Finlay and Wilkinson	-0.3	-	-
	Static stability	-0.6 ***	0.2	-
	Wricke's eco-valence	-0.3	0.0	0.6 ***

PLHT plant height; GY grain yield; * significant at the 0.05 probability level.; *** significant at the 0.001 probability level.

5.3 Grain Yield

Combined analysis of variance revealed significant variation among genotypes and herbicide treatments (Table 2). Significant GE and Genotype \times Herbicide treatment interactions were also observed for grain yield indicating that the genotypes responded differently to different environments characterized by different herbicide treatments, seasons, and years (Table 2). The h^2_{GY} was average (0.40) ranging from nearly zero in three environments of Marchouch 2015/2016 (environment D, E, F) and in Terbol 2018/2019-metribuzin treatment (environment J) and Terbol 2018/2019-no herbicide treatment (environment L) to 0.5 in three environments of Marchouch 2014/2015 (environment A, B, C) (Table 4). This indicates diverse response of each accession to the different herbicide treatments and across the different locations-seasons combinations.

In addition, Genotype \times Herbicide Treatment and GE interactions were highly significant indicating that the accessions performed differently under different herbicide treatments and in different locations and seasons (Table 2). Furthermore, Figure 2 indicated that some accessions yielded more under metribuzin and imazethapyr than under no herbicide application. This is also shown by the down trends of each herbicide applications.

The average grain yield was lower in environments treated with herbicides than in environments with no herbicide treatment at both locations in all seasons (Table 3). Figure 2 showed that the non-treated plants tend to have the highest grain yield, followed by the plants treated with metribuzin and then by those treated with imazethapyr. However, the tolerant and moderately tolerant accessions (FB2601 and IG13530) to both herbicides yielded more under metribuzin than under no herbicide treatment across environments, and the tolerant accessions (IG70622 and

FB2528) yielded similarly under imazethapyr treatment and under no herbicide treatment across environments.

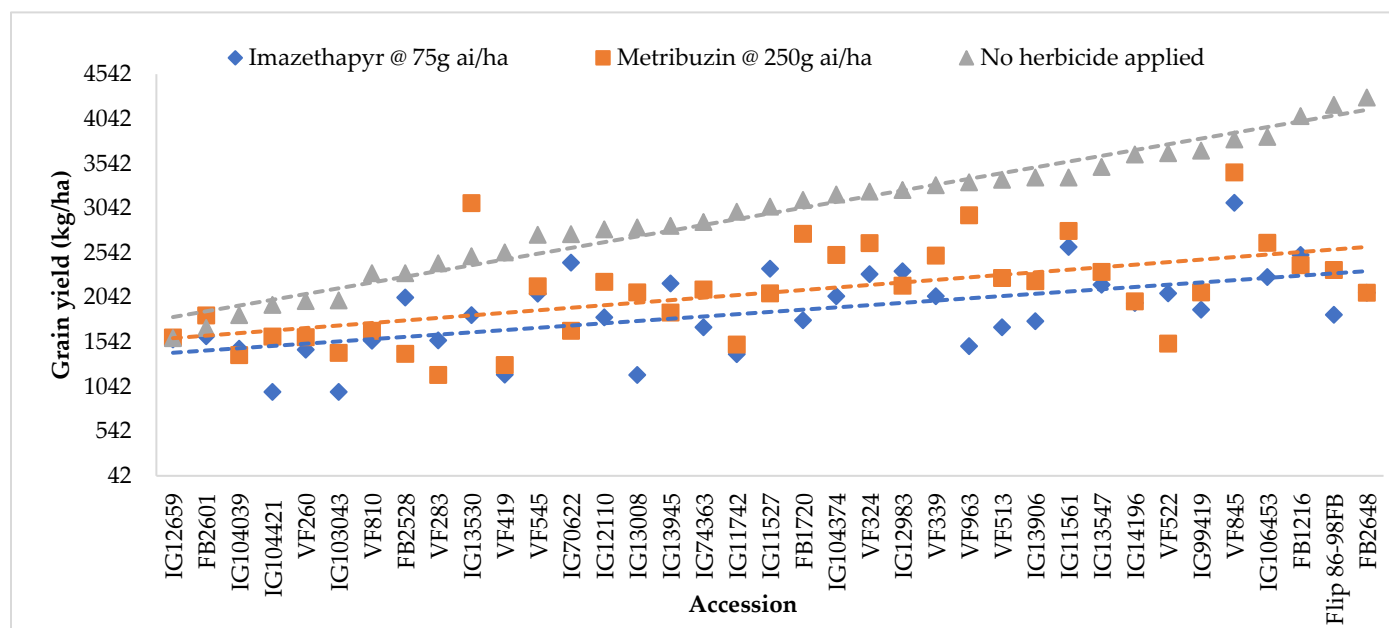


Figure 2. Mean grain yield (kg/ha) of studied accessions across seasons–locations under imazethapyr, metribuzin and no herbicide application treatments (SE = 38.76).

As significant GE interactions were detected for grain yield, the grain yield stability was assessed based on the same parameters used to evaluate the plant height stability as presented in Table 6. Considering the cultivar superiority and the ability of a genotype to have an above average mean performance across environments, VF845 had the best grain yield performance in all environments. As for the ability of the genotypes to maintain a stable performance across environments FB2528 was considered the most stable based on the static stability parameter, VF810 based on Wricke’s eco-valence, as it had the lowest eco-valence value, and IG103043 based on Finlay and Wilkinson stability parameter, as they received the lowest values of these parameters.

Table 7. Cultivar superiority index, static stability, Wricke’s eco-valence and Finlay and Wilkinson values for the grain yield of faba bean accessions evaluated in 12 different environments. Values marked in bold belong to the 10 most stable accessions, while values presented between brackets reflect their ranking.

Accession	Accession Number	Cultivar Superiority	Static Stability	Wricke’s Eco-valence	Finlay-Wilkinson
IG11561	1	22,485(9)	8927(13)	29,797(3)	0.3013(17)
IG12110	2	28,697(15)	12,597(19)	38,689(6)	0.2918(23)
VF283	3	48,317(33)	3891(5)	36,277(5)	0.2909(28)

IG13906	4	26,485(14)	13,087(22)	73,390(22)	0.2973(30)
IG74363	5	38,191(26)	13,269(23)	70,087(19)	0.2864(5)
IG13530	6	31,653(20)	13,365(24)	69,391(18)	0.2972(27)
IG13547	7	14,591(2)	16,564(28)	88,280(27)	0.3218(21)
VF513	8	22,008(8)	22,278(31)	126,327(33)	0.2951(29)
VF522	9	33,940(23)	9839(16)	80,135(24)	0.2838(22)
IG11742	10	48,025(32)	12,793(20)	62,918(14)	0.3075(11)
IG12659	11	41,996(29)	8638(12)	128,754(34)	0.2864(34)
VF419	12	50,741(34)	2913(2)	28,269(2)	0.2863(16)
IG104039	13	43,884(30)	9326(14)	135,959(35)	0.3008(24)
FB2648	14	24,007(11)	24,953(34)	119,856(31)	0.2838(35)
FB2528	15	40,875(28)	2728(1)	57,220(12)	0.3219(3)
FB2601	16	56,023(36)	3523(3)	106,197(30)	0.2833(10)
IG104374	17	31,852(21)	15,414(27)	90,573(28)	0.3029(2)
IG104421	18	60,697(37)	5081(6)	63,444(15)	0.3014(32)
IG106453	19	20,382(5)	12,874(21)	72,541(21)	0.2953(7)
Flip 86-98FB	20	20,704(6)	13,909(25)	49,157(9)	0.2921(20)
IFB1216	21	17,744(3)	16,801(29)	91,972(29)	0.2975(31)
IG11527	22	25,508(13)	6357(9)	34,188(4)	0.2908(9)
VF845	23	4164(1)	23,917(33)	255,879(37)	0.2882(26)
IG99419	24	30,080(18)	19,235(30)	82,221(25)	0.2918(13)
VF324	25	24,172(12)	5996(8)	70,922(20)	0.3094(14)
VF339	26	28,852(17)	8030(11)	46,802(7)	0.2951(15)
VF963	27	20,374(4)	ND	73,431(23)	0.284(12)
IG13945	28	28,731(16)	7384(10)	58,930(13)	0.2695(25)
IG14196	29	31,459(19)	15,412(26)	66,326(17)	0.2951(33)
FB1720	30	40,017(27)	10,146(17)	123,054(32)	0.3219(8)
IG13008	31	31,970(22)	23,600(32)	144,645(36)	0.2974(4)
IG103043	32	44,948(31)	ND	49,900(10)	0.2833(1)
IG70622	33	37,920(25)	9628(15)	55,610(11)	0.286(37)
VF545	34	23,971(10)	ND	64,208(16)	0.2917(19)
IG12983	35	21,023(7)	10,532(18)	46,866(8)	0.2908(18)
VF810	36	36,277(24)	5569(7)	15,481(1)	0.2953(6)

VF260	37	54,013(35)	3550(4)	85,871(26)	0.1424(36)
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Among the genotypes that had grain yield above average in all environments, IG12983, which was tolerant to metribuzin and imazethapyr, was the only one that was able to maintain a stable performance across environments; it was identified as stable by Wricke's eco-valence. Wricke's eco-valence was the only effective parameter in identifying a stable and high yielding genotype, unlike the static stability and Finlay and Wilkinson parameters that identified mostly genotypes with low grain yield as being the most stable.

The correlation between the stability parameters is shown in Table 6. The correlation coefficient varied between -0.6 and 0.6 . This result indicates inconsistency in the classification between the parameters. Highly significant and negative correlation (-0.6) was observed between static stability and Finley and Wilkinson's parameter and highly significant and positive correlation was observed between static stability and Wricke's eco-valence (0.6). However, even with strong correlation between methods, genotype ranking can be different between the methods. IG11527 and VF810 had the most stable grain yield as they ranked among the 10 most stable accessions by different stability parameters at the same time.

5.4 GGE Analysis

The GGE-biplot presented in Figure 3 accounted for 68.23% and 76.37% of the total variability for the plant height and grain yield, respectively. The environments with low narrow sense heritability values were excluded from the GGE biplot analysis as most of the variations observed are not genetic and might be related to the environmental conditions. The values of narrow sense heritability obtained in the present study shows that the terminal drought at Marchouch 2014/2015 and high rainfall at Terbol 2018/2019 might have led to low values and therefore the corresponding environments (D, E, F, J, K and L) were excluded from the analysis.

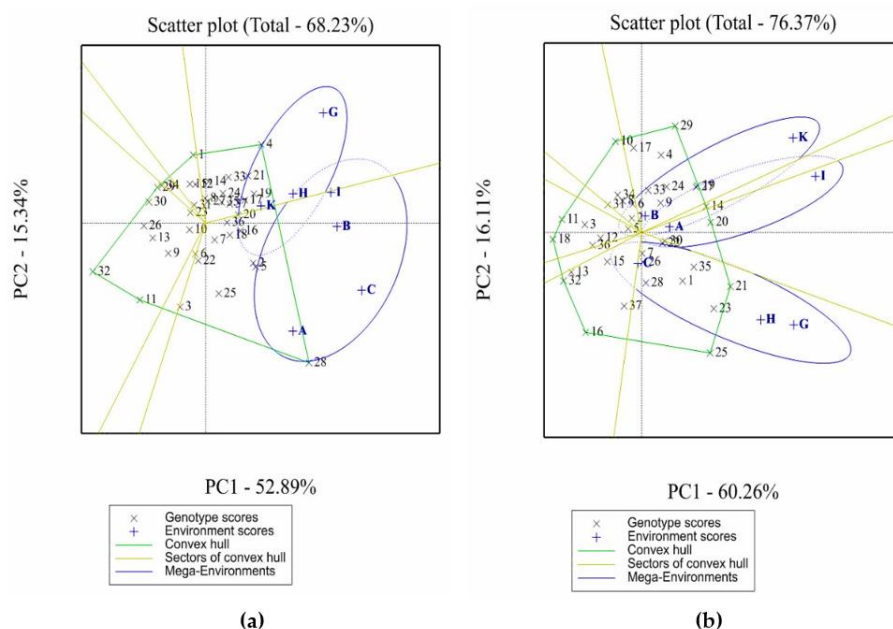


Figure 3. GGE-biplot showing the performance of each faba bean genotype in each environment and the “who-win-where” pattern of genotypes and environments for plant height (a) and grain yield (b).

5.4.1 Evaluation of Test Environments

GGE-biplot provides a summary of the relationship between test environments. Two environments are positively correlated if the angle between their vectors is less than 90° [26]. Based on this, the plant height biplot (Figure 3a) showed positive and high correlation between two imazethapyr @75 g ai/ha treatments of Terbol 2015/2016 and Terbol 2018/2019 (environments H and K) and the non-treated treatment of Terbol 2015/2016 (environment I). However, there was low correlation between environments A (metribuzin 250 g ai/ha-Marchouch 2014/2015) and G (metribuzin 250 g ai/ha-Terbol 2016/2017) as the angle between the two corresponding vectors was nearly 90° .

On the other hand, the grain yield biplot (Figure 3b) shows positive and high correlation between the non-treated treatment Terbol 2015/2016 (environments I) and the metribuzin 250 g ai/ha of Marchouch 2014/2015 (A) and between the metribuzin 250 g ai/ha of Terbol 2015/2016 (G) and the imazethapyr 75 g ai/ha of Terbol 2015/2016 (H). However, the correlation between imazethapyr 75 g ai/ha (B) and non-treated treatment of Marchouch 2014/2015 (C) indicated no association.

GGE biplots (Figure 3) also provide information about the discriminating ability of each test environment for plant height and grain yield. Figure 3a shows that, among the test environments, 250 g ai/ha metribuzin of Terbol 2015/2016 (environment A) and non-treated

treatments of Terbol 2015/2016 (C) and Marchouch 2014/2015 (G) had the longest vector and hence were highly discriminating for plant height evaluation. On the other hand, metribuzin 250 g ai/ha (G) and non-treated treatment of Terbol-2015/2016 (I) and imazethapyr 75 g ai/ha treatment of Terbol-2018/2019 (K) were highly discriminating for grain yield evaluation (Figure 3b). Moreover, the least discriminating environments are those having the shortest vectors. Based on this, imazethapyr 75 g ai/ha treatments of Terbol 2015/2016 and Terbol 2018/2019 were identified as the least discriminating environments for plant height evaluation while three environments of Marchouch 2014/2015 were identified as the least discriminating environments for grain yield evaluation.

5.4.2 Identification of Mega-Environments and Specific Adapted Accessions.

The plant height GGE biplot (Figure 3a) was divided into 6 sections where the 12 environments fell into two of these, and accordingly two mega-environments were identified. The first mega-environment contained three environments of Marchouch 2014/2015 (environments A, B and C), and the non-treated treatment of Terbol 2015/2016 (I). The second mega-environment includes environments G (metribuzin 250 g ai/ha treatment-Terbol 2015/2016), H (imazethapyr 75 g ai/ha-Terbol 2015/2016) and K (imazethapyr 75 ai Terbol 2018/2019). IG13945 (28) and IG13906 (4), which are moderately tolerant or tolerant to metribuzin and imazethapyr, were the tallest genotypes in the first and in the second mega-environments, respectively. The GGE biplot for grain yield (Figure 3b) was divided into 6 sections where the 12 environments fell into three sections and accordingly three mega-environments were identified. The first mega-environment had imazethapyr 75 g ai/ha of Marchouch 2014/2015 (B) and Terbol 2018/2019 (K); the second one had 250 g ai/ha metribuzin of Marchouch 2014/2015 (A) and non-treated treatment of Terbol 2015/2016 (I) while the third mega-environment had metribuzin 250 g ai/ha (C) and imazethapyr 75 g ai/ha (G) of Terbol 2015/2016 and non-treated treatment of Marchouch 2014/2015 (H). IG106453 (19) had the highest yield in the first mega environment, FB2648(14) in the second and FB1216 (21) in the third mega environments.

5.4.3 Performance of Tested Accessions

Figure 4a, b show the ranking of genotypes based on plant height and grain yield performance and stability in 12 environments. The mean yield performance and stability of genotypes were evaluated using an average tester axis (ATA) that passes through the origin [27,29]. Based on Figure 4a, 17 accessions were shorter than the average plant height across the 7 environments as they are located on the left side of ATA. The other 20 accessions were taller than the average across the 7

environments as they are located on the right side of ATA; 7 of them were tolerant to both herbicides' treatments across environments.

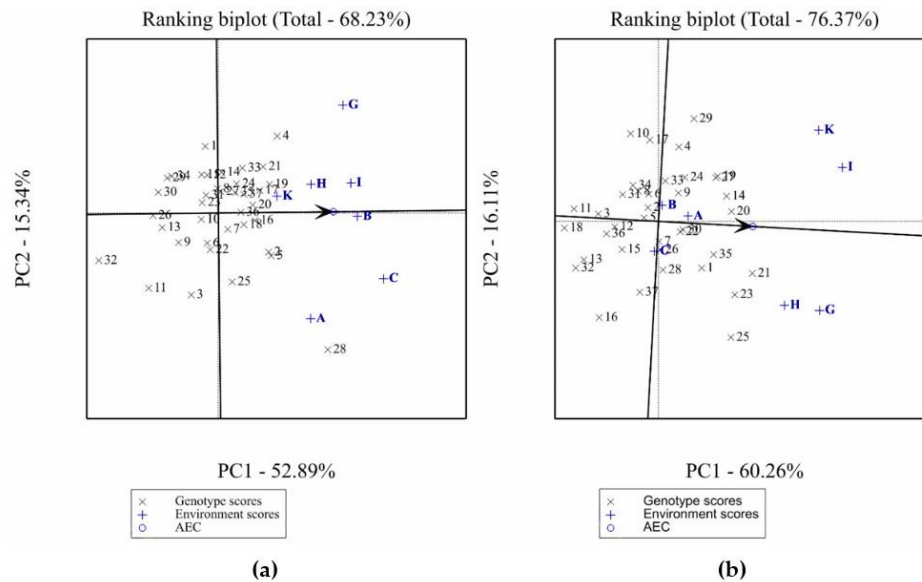


Figure 4. Ranking plot of the GGE showing the performance and stability of the faba bean genotypes for plant height (a) and seed yield (b).

Figure 4a shows that among the accessions tolerant to both herbicides with plant height above the average, FB2601 (16), IG104421 (18) and IG12983 (35) were the most stable as they had the shortest projection to the ATA. On the other hand, despite being moderately tolerant/tolerant to metribuzin and imazethapyr with good plant height performance across seven environments, IG13945 (28) was the least stable among all the accessions given that it had the longest projection to the ATA.

Regarding grain yield performance, Figure 4b shows that 18 accessions yielded less than the average grain yield across the seven environments as they are located on the left side of the ATA. The remaining 19 accessions yielded more than the average grain yield as they were located on the right side of ATA; 6 of them were tolerant to both herbicides' treatments across environments. Among the accessions that yielded more than the average grain yield and are tolerant to both herbicides, FB2648 (14), IG12983 (35) and VF522 (9) were the most stable as they had the shortest projection to the ATA. Hence, based on Figure 4 a, b, the metribuzin and imazethapyr tolerant accession IG12983 (35) was considered a superior genotype in terms of plant height and grain yield as it showed a good and stable performance for both traits across environments. Furthermore, the

following accessions VF963 (27), FB2648 (14), and IG70622 (33) that are tolerant to both herbicides performed well and were moderately stable across the 7 environments.

6 Discussion

Multi-environment trials are an integral part of the breeding pipeline to better understand the performance of tested accessions under a wide range of environmental conditions and therefore breeders are able to characterize the mega environments and identify cultivars adapted to specific environments or with broad adaptability [20,30]. In this study, faba bean accessions were evaluated in a wide range of environmental conditions created by different site–season–herbicide treatment combinations.

Faba bean accessions flowered and matured earlier in the environments with no herbicide treatment than in the environments with metribuzin or imazethapyr treatments in all sites and seasons. Past studies also reported a delay in flowering and maturity time of different legume crops treated with metribuzin or imazethapyr [31,35]. This delay might be the result of the growth inhibition of the crops amid their treatment with herbicide [36,38]. Faba bean accessions flowered and matured earlier in Marchouch than in Terbol. This might be attributed to cooler and more rainy weather in Terbol as compared to Marchouch. Past studies also reported decline in crop duration under heat and drought conditions in faba bean [39,40], lentil [41,42], chickpea [43], and common bean [44]. The earlier onset of flowering and maturity was observed in the non-treated environment of Marchouch 2016–2017 season; this was expected as it resulted from a combination of an exceptional warm and dry season and no herbicide treatment.

Plant height and grain yield were higher in the environments with no herbicide treatment than in the environments with metribuzin or imazethapyr treatments in all sites and seasons. Several studies also reported reduction in plant height and grain yield of accessions treated by herbicides in chickpea [34,45] and lentil [31,46]. This reduction might be due to the growth and photosynthesis inhibition caused by both metribuzin and imazethapyr [36,38,47]. Plant height and grain yield were higher in Terbol clustered under high rainfall than in Marchouch with low rainfall conditions. The highest average plant height and grain yield were recorded in the experiments with no herbicide treatments at Terbol 2018–2019; this was expected as it resulted from a combination of lowest temperatures, highest rainfall conditions and no herbicide application.

The heritability estimates are effective when combining data from diverse environments as the phenotypic value used to estimate the heritability is the mean value obtained across experiments

and replicates [48]. In our study, heritability estimated for grain yield was highly affected by the stress environments followed by those estimated for plant height, and days to flowering and maturity. Similar observations were reported for heritability in faba bean by Toker [15]. Mohamed [49], Abdelmula et al. [50], Ceccarelli [51], and Atlin and Frey [52] concluded that lower heritability was expected in low-yielding environments. Therefore, the selection of faba bean genotypes is best done under optimum environments that are less likely to encounter stress-periods. Furthermore, moderate to high values of narrow sense heritability reported in the present study are important because the response to selection depends on the additive genetic variance captured by the narrow-sense heritability [53] and therefore they make a good basis for further genetic analysis and allow for true replication of a genotype in and across multiple environments [48,54].

Breeding programs focus on the evaluation of the performance and stability of accessions that have traits of economic importance under diverse environments. The stability analysis conducted in the present study allowed the identification of stable and high yielding genotypes across different environmental conditions. A stable genotype should have an above average and stable performance across environments [22,23]. The various stability parameters used in this study ranked plant height and grain yield of genotypes differently at different test environments. The inconsistency in ranking of cultivar superiority, Finlay and Wilkinson and Wricke's eco-valence indices were also reported in faba bean [21], pearl millet [55] and maize [56]. Our results agree with the results reported by Mustapha and Bakari [57] who observed no similarity between static and cultivar superiority but are contrary to the ones reported by Dehghani et al. [58] who observed similarity between Finlay and Wilkinson and cultivar superiority when ranking lentil genotypes.

Our results suggest that some genotypes had stable plant height and grain yield performance based on more than one parameter, but their rankings differed with each parameter. This implies that the comparisons may greatly depend on the parameter used as also observed by Milioli et al. [59] and Westcott [60] and thus more than one parameter should be used to characterize and explore performance of genotypes across environments and enable more reliable selection and recommendation of genotypes [61].

Our results also suggest that the selection for genotypic performance stability based on the static stability, Wricke's eco-valence and Finlay-Wilkinson parameters favor genotypes having plant height and grain yield lower than the population averages. Similarly, Temesgen et al. [21] and Fikere et al. [62] also reported that low-yielding faba bean and lentil genotypes were more stable than high-yielding ones.

Static stability was highly correlated with two other stability parameters for both plant height and grain yield. Seife and Tena [63] found that Wricke's eco-valence was positively correlated with all stability parameters. However, selecting genotypes based on this method exclusively may not be suitable to identify faba bean accessions that are high-yielding and stable. The use of the Finlay and Wilkinson parameter and Static stability as a selection tool would favor superior and stable genotypes for plant height and grain yield, respectively. Temesgen et al. [21] identified high yielding genotypes that show static stability despite the finding that both high yield and static stability rarely occur in multi-location trials. The classification of low yielding genotypes as stable and high yielding genotypes as unstable by the different stability parameters might be due to the type of accessions evaluated. In the present study, the evaluated accessions are pure lines that have narrow genetic base, narrow adaptability and generally are low yielding and unstable due to homozygosity [64,65].

Plant breeders routinely conduct GGE biplot analysis of multi-environment trials to identify ideal test locations, to reduce the cost of breeding and testing strategies, and to identify genotypes that are widely or specifically adapted. The partitioning of the total sum of squares through GGE biplots obtained in our study shows that there were differential plant height and grain yield performances of faba bean genotypes across environments and consequently a high GE interaction. This interaction could reduce the accuracy of genotype evaluation and selection process [66]. Many GGE studies have been carried out in faba bean and other crops but none of them covers the effect of herbicide treatments. The present study employed a GGE biplot to analyze data from multi-location trials carried out across different locations and under different herbicide treatments over three years. Herbicide application is greatly influenced by weather conditions [67,68,69] and therefore evaluation of the environments and genotypes with herbicide treatment is pertinent to identify genotypes with stable herbicide tolerance.

The GGE biplot was used to evaluate the test environments. An environment is considered ideal for genotype testing when it discriminates the genotypes and represents the environments [16]. The presence of correlation between two environments means that similar information about the genotype performance is derived from them [23] and therefore could be an option to reduce the number of test environments and, as a result, to establish a cost-effective breeding program. The correlations observed in our study between two environments are reliable as both plant height and grain yield biplots accounted for more than 60% of the total variation [29]. Yang et al. [70] claimed that a GGE biplot is considered useful if the two PCs account for more than 60% of the (G+GE)

variability. As the GGE biplot that included all the environments accounted for only a small percentage (less than 60%) of the total variability, the patterns obtained were considered less useful and a more reliable and informative GGE biplot was obtained after excluding the low heritable environments.

According to Yan and Tinker [26], the test environments that are less discriminating provide little information on the genotype differences and should not be used as test environments. Hence, in this study among the seven test environments, environment G (metribuzin treatment of Terbol 2015/2016) is the ideal environment for plant height evaluation of genotypes as it is highly correlated with other environments (K, I, H, and B) and is highly discriminating. On the other hand, the ideal environment for evaluating grain yield of faba bean genotype is also environment G (metribuzin treatment of Terbol 2015/2016) as it is highly correlated with many other environments (H, A, B and C) and is highly discriminating. Our results suggest that the discriminating ability of environments was highly influenced by the weather conditions as the three environments of Marchouch 2016–2017 where the weather was exceptionally warm and dry were the least discriminating for grain yield evaluation and therefore, when choosing the testing sites for herbicide tolerance it is better to choose a site that is less likely to have a warm and dry spell period during the growing season like Terbol station.

GGE biplot is an effective visual tool for identifying the mega-environment issues and showing the specific adaptation of the genotypes and which cultivar won in which environments [29,71]. A mega-environment is defined as a group of locations that consistently share the same best cultivar(s) [27]. “Which-won-where” plots constructed in the present study grouped the test environments that represent a combination of season–location-herbicide treatment into different mega-environments. However, the grouping did not correspond with the geographic location or herbicide treatment applied; the grouping seemed to be influenced by the weather conditions and the non-repeatability of the winning genotype in the same geographic location or under same herbicide treatments might be the result of the weather fluctuations observed in the same location from one season to another and to the effect that the weather conditions have on the efficiency of herbicide treatments.

To delineate a mega-environment, the consistency of the genotype’s performance and the repeatability of the winning genotype in the same locations are necessary [72]. The reason for not meeting this condition in our study might be because plant height and grain yield biplots couldn’t capture all the GE variation. Mega-environments are homogeneous groups of locations that reduce

research costs by enabling fewer representative environments to be selected for genotype evaluation [73]. However, the identification of mega-environments is not easy. Many studies identified mega-environments in faba bean for grain yield and chocolate spot (*Botrytis fabae*) disease resistance [74], autumn or spring sowing adaptation [20,30] and resistance to Ascochyta blight (*Ascochyta fabae*) [75] and many other studies attempted to define mega-environments in spring wheat [76], sugarcane [77] and rice [78]. Our study is the first attempt to identify mega environments that englobe diverse herbicide treatments.

An ideal genotype is defined as one of the highest yielding across the test environments and is stable in performance [16]. The metribuzin and imazethapyr tolerant accessions which showed a good and stable plant height and grain yield performances in the current study are promising and of great importance for faba bean growers. As plant height is highly correlated with the biological yield [39,79], the accessions showing high and stable plant height performance are very important for the regions where faba bean is grown mainly for animal feeding such as Ethiopia and the Mediterranean countries. The environments evaluated in the present study represented different herbicide treatments applied in different sites and seasons. Hence, the genotypes mentioned are less sensitive to the environmental changes, have high yield, and are tolerant to metribuzin and imazethapyr treatments. Some of the high yielding faba bean genotypes reported in the present study were sensitive to environmental changes. Similar results were also obtained in other crops such as soybean [80,81].

7 Conclusions

The present study shows significant Genotype \times Environment interaction for grain yield and plant height which highlights the need to select genotypes well adapted to specific environment as well as broadly adapted genotypes. The performance and stability of faba bean genotypes were analyzed using four different stability parameters. These stability parameters showed inconsistency in the ranking of genotypes and showed that different stability parameters tend to favor low yielding genotypes. These parameters may not be appropriate, as both breeders and farmers prefer to adopt genotypes that are high-yielding and at the same time perform consistently across environments. GGE identified IG12983 as a superior genotype in terms of plant height and grain yield as it showed a good and stable performance for both traits across locations, seasons, and herbicide treatments which make it attractive to the farmers as it can provide an effective weed management tool. Some accessions had specific adaptation to at least one of the defined mega environments but not to

others; IG13945 and IG13906 were specifically adapted to one mega environment in terms of plant height while IG106453, FB2648 and FB1216 were specifically adapted to one mega environment in terms of grain yield. Moreover, some accessions were high yielding but unstable across environments while others were low yielding and stable. To develop superior herbicide tolerant cultivars that are broadly adapted to different mega environments, there is a need to cross the tolerant germplasm identified in the present study with other cultivars adapted to specific environments. These lines could also be crossed with other cultivars to accumulate traits with economical interest in new faba bean varieties. Furthermore, this study suggests conducting herbicide screenings under environments that are less likely to experience drought to avoid the confounding effect of herbicides and drought.

8 References

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9 Supplementary Materials

Table S1. Faba bean accessions with different degree of tolerance to Metribuzin and Imazethapyr used in the present study.

Accession number	Accession name	Origin	Response to Metribuzin @ 250 g ai/ha	Response to Imazethapyr @ 75 g ai/ha
1	IG11561	Algeria	T	T
2	IG12110	Algeria	T	T
3	VF283	Bulgaria	T	MT
4	IG13906	Canada	MT	T
5	IG74363	Canada	T	MT
6	IG13530	Cyprus	MT	T
7	IG13547	Cyprus	T	MT
8	VF513	Egypt	MT	T
9	VF522	Egypt	T	T

10	IG11742	Ethiopia	T	MT
11	IG12659	Ethiopia	MT	MT
12	VF419	Ethiopia	MT	MT
13	IG104039	Ethiopia	T	T
14	FB2648	Ethiopia	T	T
15	FB2528	France	T	T
16	FB2601	France	T	T
17	IG104374	Germany	MT	T
18	IG104421	Germany	T	T
19	IG106453	Greece	MT	T
20	Flip 86-98FB	Lebanon	MT	T
21	FB1216	Netherlands	MT	T
22	IG11527	Pakistan	MT	MT
23	VF845	Peru	MT	T
24	IG99419	Portugal	MT	T
25	VF324	Russia	MT	T
26	VF339	Russia	MT	T
27	VF963	Spain	T	T
28	IG13945	Sudan	MT	T
29	IG14196	Sudan	MT	T
30	FB1720	Switzerland	MT	T
31	IG13008	Syria	MT	T
32	IG103043	Syria	MT	MT
33	IG70622	Syria	T	T
34	VF545	Tunisia	T	MT
35	IG12983	Tunisia	T	T
36	VF810	United Kingdom	MT	T
37	VF260	Unknown	T	MT

S susceptible, R resistant, MT moderately tolerant, according to Abou-Khater et al., 2021 and ICARDA, unpublished data.

CHAPTER IV

Genomic regions associated with herbicide tolerance in a worldwide faba bean (*Vicia faba* L.) collection

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1. Abstract

Weeds represent one of the major constraints for faba bean crop. The identification of molecular markers associated with key genes imparting tolerance to herbicides can facilitate and fasten the efficient and effective development of herbicide tolerant cultivars. We phenotyped 140 faba bean genotypes in three open field experiments at two locations in Lebanon and Morocco against three herbicide treatments (T1 metribuzin 250 g ai/ha; T2 imazethapyr 75 g ai/ha; T3 untreated) and one in greenhouse where T1 and T3 were applied. The same set was genotyped using genotyping by sequencing (GBS) which yield 10,794 high quality single nucleotide polymorphisms (SNPs). ADMIXTURE software was used to infer the population structure which revealed two ancestral subpopulations. To identify SNPs associated with phenological and yield related traits under herbicide treatments, Single-trait (ST) and Multi-trait (MT) Genome Wide Association Studies (GWAS) were fitted using GEMMA software, showing 10 and 14 highly significant associations, respectively. Genomic sequences containing herbicide tolerance associated SNPs were aligned against the NCBI database using BLASTX tool using default parameters to annotate candidate genes underlying the causal variants. SNPs from acidic endochitinase, LRR receptor-like

serine/threonine-protein kinase RCH1, probable serine/threonine-protein kinase NAK, malate dehydrogenase, photosystem I core protein PsaA and MYB-related protein P-like were significantly associated with herbicide tolerance traits.

2. Introduction

Faba bean (*Vicia faba* L.), also known as broad bean, fava bean, horse bean and field bean, was first domesticated in the Near East around 9000–10,000 BC^{1,2,3}. The recent estimates suggest that it is extensively grown on 2.57 M ha area distributed across 38 countries with global production of 5.4 million tonnes⁴. Faba bean is an important source of food and feed for human and animal consumption because its seeds are rich in proteins, carbohydrates, fibers and micronutrients⁵. Faba bean plays an important role in sustainable agriculture and ecosystem services because of its ability to improve soil fertility by fixing atmospheric nitrogen⁶, and its potential to enhance the grain yield of succeeding/companion crops when planted in rotation or intercropped with cereals⁷. Concerted efforts have been undertaken to improve yield, adaptation to different environments, tolerance to abiotic stresses including heat, drought, waterlogging and frost^{8,9,10} resistance to biotic stresses such as diseases, insect pests, viruses and parasitic weeds^{11,12}, seed quality¹³ and other agronomic traits. These efforts have more than doubled the global average yield from 0.9 tonnes/ha in 1964 to 2.1 tonnes/ha in 2019⁴. However, the current production remains insufficient to meet its global consumption. Faba bean performance is highly influenced by environments and genotype × environment (GE) interaction, making phenotypic selection for quantitative traits of breeders' interest inefficient and cumbersome.

Faba bean has a relatively large genome size of 13 Gb¹⁴. Thanks to the advances in the next generation sequencing technologies (NGS) that has enabled the generation of large volumes of sequences^{15,16,17} and facilitated the discovery of single nucleotide polymorphisms (SNPs) that can be associated with key breeding traits either through biparental mapping or through genome wide association studies (GWAS)^{15,18}. Unlike biparental mapping, GWAS utilizes natural populations and exploits linkage disequilibrium (LD) to detect SNP-trait associations with higher resolution¹⁹. However, the power of GWAS depends on the size and structure of the population used for the analysis²⁰. While it is sometimes not feasible to phenotype large populations in a single field trial, multiple field trials, e.g. different treatments, locations or seasons, can be jointly analyzed in one model named as multi-variate or multi-trait GWAS which has shown to have higher power compared to the standard single-trait GWAS²¹. Such approach can assist conventional breeding by

implementing marker assisted selections in early generations^{18,22}. Although significant progress has been made in faba bean genomics and many genetic maps are available^{23,24,25}, the marker density of most of them is still too low to enable accurate prediction of desired traits. SNPs correlated with traits of interest such as resistance to ascochyta and broomrape or vicine-convicine content^{26,27,28,29} have been identified, however, no study was conducted to associate SNPs with herbicide tolerance in faba bean.

Weeds are among the difficult-to-control biotic stresses that affect faba bean³⁰. When weeds are left uncontrolled, they cause severe loss on grain yield of up to 70%³¹. An integrated approach with many control measures has been recommended to provide protection against weeds^{32,33,34} but with limited success. Many studies have acknowledged breeding for weed resistance by selecting for morphological characteristics that promote competition and allelopathy such as early seedling emergence, seedling growth, greater plant height, greater root volume^{35,36,37}, but the resistance against most parasitic weeds is a difficult task because of its complex nature and low heritability^{38,39}. Thus, recent studies have focused on developing herbicide tolerant faba bean lines^{40,41}. Abou-Khater et al.⁴² evaluated faba bean germplasm for traits associated with tolerance to metribuzin and imazethapyr, two herbicides commonly available that can control the majority of weeds threatening faba bean production. They found that crop phenology, plant architecture and grain yield related traits were greatly affected by the herbicide treatments. Although useful sources for herbicide tolerance were identified by the authors, such field techniques are very laborious and require multi-environmental data. Associating the herbicide tolerance related traits⁴² with molecular markers to select for herbicide tolerance would facilitate the detection of useful markers that can be used to select herbicide tolerant lines in early generations. Keeping this in mind, the present study was undertaken to identify candidate loci significantly associated with tolerance to two post emergence herbicides, namely metribuzin and imazethapyr under different environments using GWAS and to identify associated SNP markers that can be used for introgressing such traits into desired agronomic background.

3. Materials and Methods

3.1. Plant Materials

A set of 134 faba bean genotypes comprising 118 landraces from 42 countries and 16 ICARDA breeding lines that were used to establish a reference set under the Generation Challenge Program (GCP) was used for phenotyping and genotyping in the present study. Previous assessment with

Simple Sequence Repeat (SSR) markers showed that the set was genetically diverse and comprised 45 *major*, 17 *minor*, 63 *equina* and 9 *paucijuga* genotypes^{22,42}. In addition to the test genotypes, six faba bean cultivars (FLIP86-98, ILB1814, Ed-damer, Hudeiba-93, Shambat-75, SML) were included in the experiments. The seeds used in the current experiments are sourced from the reserved seeds that are multiplied each year under insect-proof cages in order to ensure purity of the evaluated accessions.

3.2. Experiments

A total of four experiments were conducted: three field and one greenhouse experiments.

3.2.1 Field experiments

A total of three field experiments were conducted at two ICARDA research stations: Marchouch (33.558°N 6.693°W, altitude 255m) in Morocco and Terbol (35.98°N, 33.88°E, altitude 890m) in the Bekaa Valley of Lebanon. Marchouch station is characterized by the semi-arid environment with a Vertisol soil, mostly silty clay, while Terbol station is characterized by cool and high rainfall winter and moderate wet spring with a deep and rich clay loam soil. Each experiment comprised three treatments applied at the pre-flowering stage: T1 - Metribuzin @250 g ai/ha, T2 - Imazethapyr @ 75g ai/ha, and T3 - No herbicide application. Faba bean genotypes were sown in rotation with cereals in mid-December at Marchouch 2014/2015, late November at Terbol 2014/2015 and 2018/2019 main seasons. Each genotype was planted in 2 m long two-row plot with 0.5 m spacing between rows. At Marchouch, the crop received 291 mm of precipitation during the cropping season in addition to 30 mm irrigation during early vegetative phase; the crop was exposed to intermittent drought and heat. 120 genotypes along with the three following cultivars FLIP86-98, ILB1814 and Hudeiba-93 were evaluated at Marchouch using Augmented design⁴². At Terbol, a total precipitation of 343 mm and 810 mm was recorded respectively during 2015/2016 and 2018/2019 cropping seasons. Supplemental irrigation (30 mm) was provided at Terbol station in 2015/2016 season during dry-spell periods, while no irrigation was provided in case of highly and well distributed rains in 2018/2019. A total of 134 genotypes were evaluated at Terbol using Alpha lattice design with 15 blocks. In 2015/2016 season, the field experiment was conducted with two replicates and FLIP86-98⁴² as check. In 2018/2019 the field experiment was conducted with 3 replicates and 14 blocks and FLIP86-98, ILB1814, Ed-damer, Hudeiba-93, Shambat-75 and SML as check cultivars.

3.2.2 Greenhouse experiment

The germplasm genotypes along with six checks (FLIP86-98, ILB1814, Ed-damer, Hudeiba-93, Shambat-75, SML) were evaluated in an alpha design with two replicates and two treatments: 250 g ai/ha of metribuzin and untreated treatment during 2017/2018 cropping season. Three seeds per pot for each genotype were sown in this experiment. Irrigation was provided regularly to maintain 100% soil water capacity in pots. Temperature inside the greenhouse was fixed at 24 to 28°C the optimal day time temperature of faba bean.

The herbicide treatments applied in all experiments are metribuzin (M, T1), imazethapyr (I, T2) and the control treatment (C, T3) in which no herbicide was applied. The doses of herbicides applied are the recommended doses as per the labels of metribuzin (Sencor: Bayer) and imazethapyr (Pursuit: BASF). Both herbicides were uniformly sprayed at the rate of 250 g ai ha⁻¹ and 75 g ai ha⁻¹ respectively at the inflorescence stage BBCH code 50⁷² for the field experiments and at the stem elongation stage BBCH code 30⁷² for the greenhouse experiment using an electric sprayer with automated flow (375 L/ha). In the field, the herbicide was sprayed early in the morning to ensure a low wind speed. Details of traits scored in each trial can be found in Table S1. Traits were coded as the environment, followed by the trait, the treatment and “RI” if the score describe a reduction index. For the multi-trait GWAS analysis, the trait name does not have the name of the environment or the treatment.

3.3 Phenotyping for herbicide tolerance

Observations (Supplementary Table S1) were recorded on days to 50% flowering (DFLR) and maturity (DMAT) on plot basis for the untreated treatment, and plant height (PLHT) and grain yield per plant (GYPLT) on three plants selected randomly for all the three treatments at Marchouch 2014/2015. At Terbol station, the following additional traits were also recorded on three plants selected randomly for the three treatments: number of pods per plant (NPPLT), number of seeds per plant (NSPLT), number of branches per plant (NBrPLT) and green canopy cover (GCC). Green canopy cover expressed as the average percentage of green coverage of three plants was quantified using the Canopeo application developed by Oklahoma State University using Matlab. Under the greenhouse conditions where temperature was controlled at optimal conditions, PLHT was recorded at flowering PLHT_1 (BBCH code 60) and pod development PLHT_2 (BBCH code 70) stages⁷². The herbicide damage score (HDS) was recorded in all the

four experiments using a 1-5 scale⁴² (Supplementary Table S2) at flowering (HDS1) and pod development (HDS2) stages. The ratio of each quantitative trait was calculated for each plot using the following formula described by Abou-Khater et al.^{42,42}:

$$RI\% = 100 - \left(\frac{T}{C} \times 100 \right)$$

where RI%, the reduction index, represents the reduction or penalty in traits of herbicides treated plots compared to the control untreated plots, T is the average of plots treated with herbicide (metribuzin or imazethapyr); C is the mean of genotypes under untreated conditions.

3.4 DNA extraction and genome by sequencing analysis

Genomic DNA was extracted from young leaf tissues for each tested genotype using the DNeasy 96 Plant Kit (QIAGEN, Valencia, CA, USA) Qiagen Plant DNA Preparation Kit. For the preparation of the GBS library, the two restriction enzymes, *PstI* and *MspI*, were used to generate fewer variation in the distribution of read depth and higher number of scorable SNPs. GBS libraries were prepared with 48 barcode adapters with 4–9 bp sequence. The single read (100 base pairs) sequencing on an Illumina HiSeq 2500 produced approximately 4 million reads per genotype. Raw read sequences were processed using TASSEL-GBS 5.0 with the default parameters⁷⁴. A faba bean sequence database was constructed using 223,801 genomic and transcriptomic faba bean sequences downloaded from NCBI and pulsedb databases (www.ncbi.nlm.nih.gov and <https://www.pulsedb.org/analysis/136>) and additional faba bean sequences constructed using the Trinity assembler from one run of the GBS files. These sequences were used as a reference to align GBS sequence tags and indexed using Bowtie2 version 2.2.4⁷⁵ Bowtie2 was used to align GBS tags to faba sequences using the–very-sensitive-local option. Resulting SNPs were filtered with 20x coverage, where SNPs with more than 15% missing data or less than 5% minor allele frequency (MAF) were removed. SNPs were named by contig base pair position.

3.5 Statistical analysis of phenotyping data

The spatial statistical model was applied for variance analysis for all quantitative data using the Automatic Spatial Analysis of Row-Column modules of Genstat 19 edition⁸⁵. Significance of variation among genotypes and treatments was assessed in terms of *P*-values. The analysis of variance (ANOVA), means of genotypes, means of treatments and interactions between genotypes and treatments were estimated with standard errors using best linear unbiased prediction (BLUP)

values using GenStat software. BLUPs were used to conduct all downstream analyses. Multi environment trials analysis (META) were conducted to evaluate variation among genotypes, treatments, and the genotype x treatment interaction across trials for the traits recorded in more than one trial. Genotype and treatment were fitted as fixed parameters while environment (year-location) were fitted as random parameter.

3.6 Genome-wide association analysis

ADMIXTURE software⁷⁷ was used to infer population structure with the number of underlying subpopulations (K) ranges between 2 and 20. The analysis was run with 100 random replicates and 20 cross validations. The most probable K was determined at the point when the average cross validation (CV) values across the 100 replicates started to increase. Single-trait (ST) and Multi-trait (MT) GWAS was fitted using GEMMA software²¹ by fitting each trait independently (for the ST analysis) or fitting all field or greenhouse records together (for the MT analysis) with the default parameters and by fitting the genomic relatedness matrix as a covariate to control for population stratification⁷⁸. Bonferroni correction was used to determine the significant threshold at $p < 0.05$ but all SNPs with $p < 1E-4$ were presented as suggestive associations. Pairwise linkage disequilibrium (LD) between associated SNPs within each trait was estimated with the r^2 statistics following Weir⁷⁹ to determine the SNPs that are associated with the same quantitative trait locus (QTL). Genomic sequences containing herbicide tolerance associated SNPs were aligned against the NCBI database using BLASTX tool using default parameters to annotate potential candidate genes underlying the causal variants.

4. Results

4.1. Phenotyping

Multiple environmental models were fitted to obtain the best linear unbiased prediction (BLUP) values for each genotype and treatment across field trials. The genotypic effects for all studied traits and reduction indexes were significant across trials at a p -value < 0.001 except for the RI_{GCC} (Table 1) indicating a wide range of genotypic variation in faba bean. Significant differences were observed among treatments for all studied traits and reduction indexes except for RI_{PLHT} , RI_{GYPLT} and RI_{NPPLT} ; while significant Genotype \times Treatment interactions were observed across trials for DFLR, DMAT, PLHT, GYPLT and NSPLT (Table 1). The Genotype \times Treatment \times Environment interactions show that the effect of herbicide treatments on the traits and reduction

indexes of the genotypes was not affected by the environment except for DFLR and NSPLT and their reduction indexes (Table 1). As for the greenhouse experiment, the DFLR, PLHT and GCC varied significantly among genotypes and treatments and significant Genotype \times Treatment interactions were observed. The reduction indexes for DFLR, PLHT and GCC varied significantly also among genotypes (Table 2). Our results showed that both herbicide treatments affected the faba bean phenology by delaying significantly the DFLR and DMAT (Tables 1, 2). In addition, the post emergence application of metribuzin and imazethapyr affected the architecture of the faba bean plants by reducing the PLHT and the GCC and increasing the NbrPLT (Tables 1, 2). Moreover, a significant reduction in the GYPLT, NPPLT and NSPLT of the genotypes treated with metribuzin or imazethapyr was observed across trials. The plant height recorded in the green house experiment at two different stages showed that the treated plants tend to recover from the herbicide effect (Tables 1, 2).

Table 1. Combined analysis performed for detecting differences among faba bean genotypes (Geno), herbicide treatments (Trt), Geno \times Trt interaction, Genotype \times Environment (Geno \times Env) interaction and Geno \times Trt \times Env interaction expressed as p-value and means \pm standard error (SE) and ranges of the genotypes under trials.

			Metribuzin 250 g ai/ha		Imazethapyr 75 g ai/ha		Control		SE
		p-value	Mean	Range	Mean	Range	Mean	Range	
DFLR (DAS)	Geno	<0.001	99.51	90.96-121.39	99.44	90.48-121.61	98.10	90.60-117.94	2.57
	Trt	<0.001							
	Geno x Trt	<0.001							
	Geno x Env	<0.001							
	Geno x Trt x Env	<0.001							
DFLR_RI	Geno	<0.001	-2.37	-10.22-3.57	-2.31	-11.90-3.95			2.71
	Trt	0.749							
	Geno x Trt	1							
	Geno x Env	<0.001							
	Geno x Trt x Env	<0.001							
DMAT (DAS)	Geno	<0.001	162.92	159.20-167.00	161.88	158.30-167.00	161.05	158.00-166.80	1.17
	Trt	<0.001							
	Geno x Trt	<0.001							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.036							
DMAT_RI	Geno	<0.001	-1.63	-4.23-0.13	-1.07	-3.14-0.13			0.86
	Trt	<0.001							
	Geno x Trt	0.22							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.970							
PLHT (cm)	Geno	<0.001	61.61	37.28-83.24	60.73	32.40-83.09	73.44	36.29-104.09	6.56
	Trt	<0.001							

PLHT_RI	Geno x Trt	<0.001							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.002							
	Geno	<0.001	13.93	-41.62-37.27	17.18	-1.16-43.90			11.36
GYPLT (g)	Trt	0.002							
	Geno x Trt	0.997	0.809						
	Geno x Env	<0.001							
	Geno x Trt x Env	0.809							
	Geno	<0.001	14.68	-2.37-36.82	14.55	-1.73-40.57	20.37	1.25-41.94	5.59
	Trt	<0.001							
GYPLT_RI	Geno x Trt	<0.001							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.190							
	Geno	<0.001	-41.56	-108.26-13.10	-46.27	-113.73-13.35			2.82
NPPLT	Trt	0.105							
	Geno x Trt	0.999							
	Geno x Env	0.103							
	Geno x Trt x Env	0.787							
	Geno	<0.001	17.41	6.56-36.72	16.99	8.65-29.18	19.43	7.62-44.13	4.76
	Trt	<0.001							
NPPLT_RI	Geno x Trt	0.587							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.437							
	Geno	<0.001	-6.98	-84.04-60.49	-4.99	-81.83-50.52			37.53
NSPLT	Trt	0.393							
	Geno x Trt	1							
	Geno x Env	<0.001							
	Geno x Trt x Env	1							
	Geno	<0.001	20.38	1.54-34.68	21.94	2.28-44.85	28.82	2.36-63.65	6.91
	Trt	<0.001							
NSPLT_RI	Geno x Trt	<0.001							
	Geno x Env	<0.001							
	Geno x Trt x Env	<0.001							
	Geno	0.016	24.38	-33.02-75.63	14.1	-53.60-60.39			31.82
NBrPLT	Trt	<0.001							
	Geno x Trt	1							
	Geno x Env	<0.001							
	Geno x Trt x Env	0.007							
	Geno	<0.001	4.16	0.68-8.25	3.31	0.98-6.12	3.14	0.77-7.12	1.46
	Trt	<0.001							
NBrPLT_RI	Geno x Trt	0.931							
	Geno x Env	ND							
	Geno x Trt x Env	ND							
	Geno	<0.001	-50.49	-308.98-53.28	-18.43	-307.77-49.14			78.70
GCC	Trt	<0.001							
	Geno x Trt	1							
	Geno x Env	1							
	Geno x Trt x Env	ND							
GCC	Geno	<0.001	29.35	-0.89-59.23	25.18	1.71-53.46	34.47	3.12-67.22	11.93
	Trt	<0.001							
	Geno x Trt	0.995							

GCC_RI	Geno x Env	ND					
	Geno x Trt x Env	ND					
	Geno	0.094	5.79	-180.80-93.46	16.79	-376.14-87.11	60.34
	Trt	0.027					
	Geno x Trt	0.954					
	Geno x Env	ND					
	Geno x Trt x Env	ND					

DFLR days to flowering, DAS days after sowing, DFLR_RI DFLR reduction index, DMAT days to maturity, DMAT_RI DMAT reduction index, PLHT plant height, cm centimeter, PLHT_RI reduction index of PLHT, GYPLT grain yield per plant, g gram, GYPLT_RI GYPLT reduction index, NPPLT number of pods per plant, NPPLT_RI NPPLT reduction index, NSPLT number of seeds per plant, NSPLT_RI NSPLT reduction index, NBrPLT number of branches per plant, NBrPLT_RI NBrPLT reduction index, GCC green canopy cover, GCC_RI green canopy cover reduction index, ND no data.

Table 2. Analysis of Variance performed for detecting differences among faba bean genotypes (Geno), herbicide treatments (Trt), and *Geno × Trt* interaction for different traits and reduction indexes, expressed as *p*-value and means ± standard error (SE) and ranges of the genotypes under different treatments in the pot trial.

		Metribuzin @250 g ai/ha			Control		
		<i>p</i> -value	Mean	Range	Mean	Range	SE
DFLR	Geno	<0.001	56.65	38.93-62.21	49.04	34.94-61.80	7.12
	Trt	<0.001					
	Geno x Trt	<0.001					
DFLR_RI	Geno	<0.001	2.27	-65.37-49.79			18.26
GCC	Geno	<0.001	5.09	-0.27-10.47	6.95	2.37-10.74	1.78
	Trt	<0.001					
	Geno x Trt	<0.001					
GCC_RI	Geno	<0.001	18.62	-237.69-104.02			40.66
PLHT_1	Geno	<0.001	15.12	2.63-26.27	25.29	11.55-42.65	4.70
	Trt	<0.001					
	Geno x Trt	<0.001					
PLHT_RI_1	Geno	<0.001	28.17	-73.02-89.70			19.2
PLHT_2	Geno	<0.001	21.23	5.16-35.67	35.90	17.97-58.62	7.26
	Trt	<0.001					
	Geno x Trt	0.004					
PLHT_RI_2	Geno	<0.001	53.78	-34.25-100.00			26.05

DFLR days to flowering, DFLR_RI DFLR reduction index, GCC green canopy cover, GCC_RI green canopy cover reduction index, PLHT_1 plant height recorded at flowering (BBCH code 60), PLHT_1_RI reduction index of PLHT_1, PLHT_2 plant height recorded at pod development (BBCH code 70), PLHT_2_RI PLHT_2 reduction index.

Our results also showed that the first (HDS1) and second (HDS2) herbicide damage scores per genotype varied from 1 to 5 across trials. Combined results of the herbicide damage scores (HDS1 and HDS2) showed that after one month of the herbicide application, 5 and 42% genotypes recovered from the damaged caused by metribuzin and imazethapyr treatments while damages in 56 and 10% genotypes exacerbated (Figure 1). The herbicide damage on the remaining genotypes remained unchanged between the first and second recording dates.

Figure 2 shows that the herbicide treatments affected differently the plant height and grain yield of the treated genotypes. The reduction in plant height and grain reduction varied between almost negligible (< 10%) and high levels (> 40%).

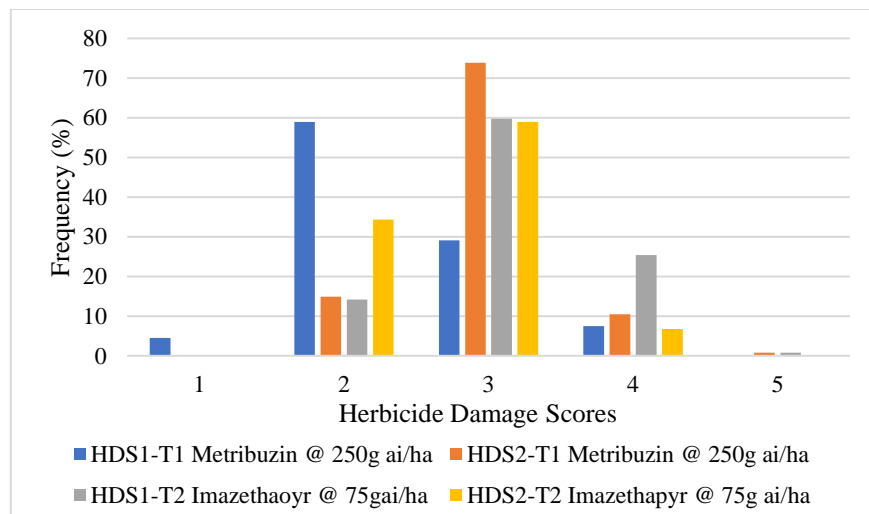


Figure 1. Distribution of faba bean genotypes for herbicide damage scores (HDS1 and HDS2) under metribuzin at 250 g ai/ha and imazethapyr at 75 g ai/ ha.

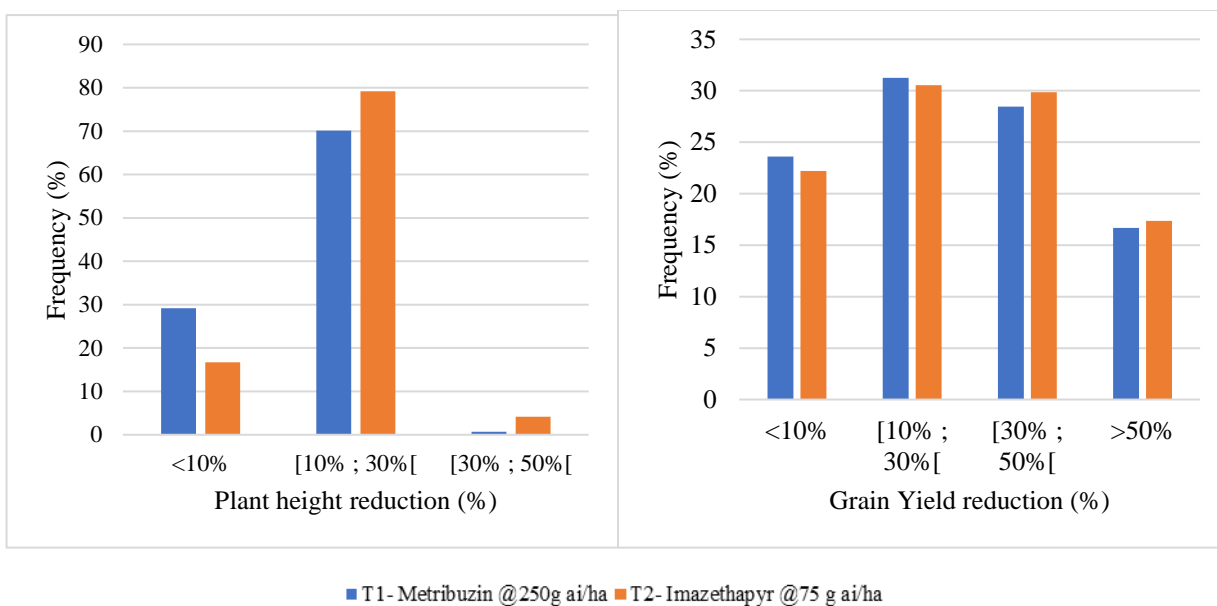


Figure 2. Distribution of faba bean genotypes for plant height reduction *PLHT_RI* (a) and grain yield per plant reduction *GYPLT_RI* (b) under metribuzin at 250 g ai/ha and imazethapyr at 75 g ai/ ha.

4.2. Genotyping and population structure

The SNP calling analysis revealed 10,794 high-quality SNPs among the studied faba genotypes. The sequence variations of these SNPs were C/T (4251 SNPs), and A/G (4029 SNPs), followed by A/T (836 SNPs), G/T (761 SNPs), A/C (619 SNPs), and C/G (298 SNPs). The average CV values for the 100 replicates of the population structure started to increase directly after $K = 2$ indicating the presence of two ancestral subpopulations in the germplasm set used in the present study. However, we presented the results of K up to 4 because their 100 replicate runs resulted in comparable classification of genotypes into ancestral subpopulations with top > 20% of replicates having almost the exact log-likelihood values (Figure 3). Beyond $K = 4$, the analysis started to output arbitrary results with inconsistent classifications even for the top 10 replicates with the highest log-likelihood values.

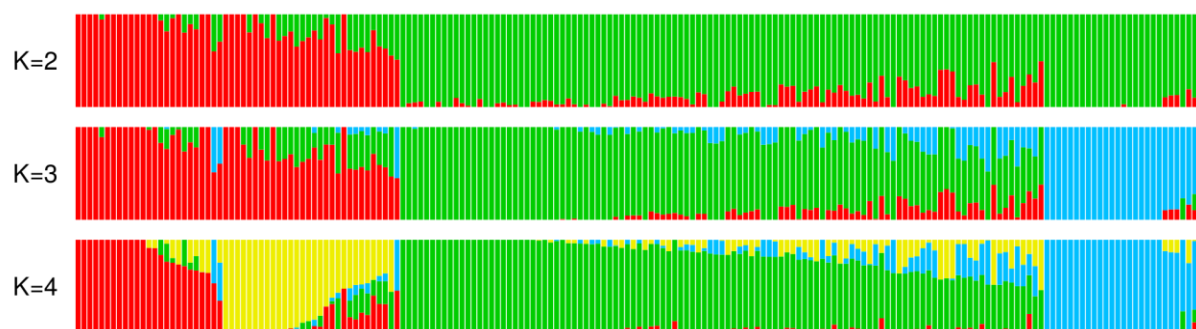


Figure 3. Population structure constructed using the SNPs data for the individual ancestry estimated using the ADMIXTURE analysis. Individuals are represented in thin vertical lines separated into segments corresponding to the assumed membership in $K = 2, 3$ and 4 genetic groups as shown by colors. Each color represents one ancestral subpopulation.

4.3. GWAS and annotation analyses

As we ended with a total of 10,794 high-quality SNPs after filtration, the Bonferroni significant threshold can be calculated as $(0.05/10,794 = 4.6E-6)$. Analyzing all 103 traits (including RI scores) with the ST-GWAS model resulted in only 10 highly significant associations with the Bonferroni threshold as well as 110 suggestive associations for only 66 traits while the remaining traits had no associated SNPs (Supplementary Table S3). These associations were represented by 105 SNPs. Only one SNP (*SNODE_27970_52*) for DFLR was associated with three treatments (I, M and C) in TR16, while another five SNPs were associated with two scores for PLHT or DFLR (Supplementary Table S3). Of these, two SNPs (*SNODE_168698_34* for PLHT with treatment I, and *SNODE_23759_68* for DFLR with treatment M in TR16) were associated with a specific trait and its correspondence RI score. Another five SNPs showed association with two different traits of which *SNODE_3696_16* and *SNODE_77186_51* were associated with GYPPLT in TR16, treatments M and I respectively, while *SNODE_22383_32* was associated with RI in TR16, treatment M, for the same traits. *SNODE_239220_75* was associated with TR19_DMAT_I and TR16_NPPT_I_RI, while *SNODE_7114_58* showed associations with five DFLR and DMAT across environments/treatments (Supplementary Table S3).

The MT-GWAS model for 20 traits across environments (including RI scores) resulted in 14 highly significant associations and 64 suggestive associations for all traits represented by 72 SNPs (Table 3). The largest number of associations (12) were detected of DMAT, while GYPPLT_RI, NPPT_RI and Score1 had the lowest number with only one association each. Most of the SNPs that showed associations with multiple traits/treatments in the ST-GWAS analysis were also detected in MT-GWAS analysis. Four SNPs showed associations with a specific trait with its

reduction index which were *SNODE_23759_68* for DFLR, *SNODE_14558_21* for NSPP, *SCONTIG73439_18* for PLHT, and *SNODE_103_72* for NBBR (Table 3). The SNP *SCONTIG127798_41* was associated with GCC and DFLR while the SNP *SNODE_22383_32* was associated with GYPPLT (Table 3).

Table 3. SNP-trait associations revealed by the MT-GWAS analysis.

Trait	SNP	allele1	allele0	MAF	P
DFLR	<u><i>SNODE_7114_58</i></u>	C	T	0.14	3.0E-07
DFLR	<u><i>SNODE_162178_22</i></u>	A	G	0.07	9.8E-07
DFLR	<i>SNODE_27970_52</i>	C	G	0.17	3.6E-05
DFLR	<i>SNODE_23759_68</i>	A	G	0.11	7.1E-05
DFLR_RI	<u><i>SNODE_23759_68</i></u>	A	G	0.10	8.6E-07
DFLR_RI	<u><i>SNODE_5725_31</i></u>	C	T	0.41	3.2E-06
DFLR_RI	<u><i>SNODE_4187_38</i></u>	G	A	0.05	3.3E-06
DFLR_RI	<i>SNODE_26501_64</i>	G	C	0.13	9.5E-06
DFLR_RI	<i>SCONTIG127798_41</i>	C	T	0.07	2.9E-05
DFLR_RI	<i>SNODE_1051_18</i>	C	T	0.08	4.3E-05
DMAT	<u><i>SCONTIG72526_35</i></u>	A	G	0.05	4.6E-06
DMAT	<i>SNODE_375879_34</i>	A	T	0.08	5.7E-06
DMAT	<i>SCONTIG93616_28</i>	C	T	0.30	1.3E-05
DMAT	<i>SCONTIG6418_84</i>	C	T	0.22	1.4E-05
DMAT	<i>SNODE_61301_40</i>	T	C	0.06	1.5E-05
DMAT	<i>SNODE_80758_20</i>	C	T	0.06	3.3E-05
DMAT	<i>SNODE_483217_44</i>	G	A	0.12	4.6E-05
DMAT	<i>SNODE_143506_34</i>	G	A	0.05	6.9E-05
DMAT	<i>SCONTIG125372_89</i>	T	C	0.05	8.3E-05
DMAT	<i>SNODE_6229_36</i>	C	T	0.11	8.4E-05
DMAT	<i>SCONTIG79953_82</i>	T	C	0.37	8.6E-05
DMAT	<i>SNODE_16244_35</i>	T	C	0.33	8.8E-05
DMAT_RI	<i>SNODE_76542_45</i>	T	G	0.06	5.1E-06
DMAT_RI	<i>SNODE_13235_37</i>	C	A	0.25	2.5E-05
GCC	<u><i>SNODE_11304_24</i></u>	A	T	0.31	3.5E-06

GCC	SCONTIG24931_19	G	A	0.06	4.4E-05
GCC	SCONTIG66488_16	G	A	0.07	7.8E-05
GCC_RI	<u><i>SCONTIG127798_41</i></u>	C	T	0.07	5.3E-08
GCC_RI	SCONTIG75553_52	T	C	0.34	8.3E-05
GCC_RI	SNODE_12134_67	T	G	0.07	9.3E-05
GYPLT	SNODE_77186_51	T	A	0.10	1.3E-05
GYPLT	SNODE_3696_16	G	A	0.11	2.5E-05
GYPLT	<u>SNODE_54972_30</u>	A	C	0.14	1.3E-07
GYPLT	<u>SCONTIG46666_46</u>	A	T	0.06	2.6E-06
GYPLT	SNODE_167460_49	C	T	0.20	6.7E-06
GYPLT	SCONTIG90061_39	A	C	0.06	8.1E-06
GYPLT	SNODE_5674_14	G	A	0.06	1.1E-05
GYPLT	SNODE_4555_43	T	A	0.11	3.8E-05
GYPLT	SNODE_34407_21	A	G	0.06	5.5E-05
GYPLT	SNODE_4363_81	C	T	0.36	7.5E-05
GYPLT	SNODE_16972_9	A	G	0.05	7.9E-05
GYPLT_RI	<i>SNODE_22383_32</i>	T	C	0.14	5.5E-05
NBrPLT	SCONTIG97891_72	A	G	0.40	7.4E-06
NBrPLT	<i>SNODE_103_72</i>	T	C	0.25	2.6E-05
NBrPLT_RI	<u>SNODE_173108_18</u>	A	G	0.06	2.6E-06
NBrPLT_RI	SNODE_2942_50	C	T	0.06	1.0E-05
NBrPLT_RI	<i>SNODE_103_72</i>	T	C	0.25	1.6E-05
NBrPLT_RI	SNODE_144193_69	C	A	0.46	9.5E-05
NPPLT	SCONTIG23347_118	G	A	0.07	2.3E-05
NPPLT	SNODE_28265_65	C	T	0.31	8.0E-05
NPPLT_RI	<u>SNODE_559376_60</u>	A	T	0.45	1.0E-11
NSPLT	<i>SNODE_14558_21</i>	A	G	0.20	1.2E-05
NSPLT	SCONTIG38056_40	C	T	0.07	2.8E-05
PLHT	<u>SNODE_43134_109</u>	A	G	0.05	2.8E-09
PLHT	<u>SNODE_134600_32</u>	T	G	0.06	3.8E-06
PLHT	SNODE_27201_27	T	G	0.06	1.0E-05
PLHT	<i>SCONTIG73439_18</i>	G	A	0.06	1.3E-05
PLHT	SNODE_78412_27	T	C	0.11	2.9E-05

PLHT	SNODE_124581_38	A	C	0.07	6.9E-05
PLHT	SNODE_113123_17	T	G	0.06	7.5E-05
PLHT	SCONTIG57859_65	G	A	0.07	7.5E-05
PLHT	SCONTIG101530_33	A	G	0.14	9.6E-05
PLHT_RI	<i>SCONTIG73439_18</i>	G	A	0.06	6.1E-06
PLHT_RI	SCONTIG157_70	T	G	0.16	9.3E-06
PLHT_RI	SNODE_14298_44	A	G	0.06	1.0E-05
PLHT_RI	SNODE_3358_54	A	C	0.18	1.1E-05
PLHT_RI	SNODE_11304_26	G	A	0.12	2.9E-05
PLHT_RI	SNODE_107804_70	A	T	0.05	3.2E-05
PLHT_RI	SNODE_176979_47	G	A	0.08	4.6E-05
PLHT_RI	SNODE_4904_26	C	T	0.17	9.2E-05
HDS1	SNODE_2908_40	G	A	0.17	4.4E-05
HDS2	SNODE_8269_115	G	A	0.07	5.8E-06
HDS2	SNODE_68619_39	G	A	0.08	1.3E-05
HDS2	SNODE_2018_104	C	T	0.07	9.9E-05
NSPLT_RI	SNODE_2107_36	C	T	0.06	6.1E-06
NSPLT_RI	SNODE_7966_59	G	A	0.06	4.2E-05
NSPLT_RI	<i>SNODE_14558_21</i>	A	G	0.20	8.7E-05

Gene annotation showed that SNP *SCONTIG127798_41* associated with reduction index of GCC and DFLR is located within a gene annotated as acidic endo-chitinase annotation, *SNODE_14298_44* associated with the reduction index of PLHT is located within a gene annotated as LRR receptor-like serine/threonine-protein kinase RCH1, *SNODE_3696_16* associated with GYPLT is located within a gene annotated as Probable serine/threonine-protein kinase NAK, *SNODE_4187_38* associated with the reduction index of DFLR is located within a gene annotated as malate dehydrogenase, *SNODE_559376_60* associated with the reduction index of NPPLT is located within a gene annotated as photosystem I core protein PsaA, while *SNODE_7114_58* associated with DFLR is located within a gene annotated as MYB-related protein P-like (Supplementary Table S4).

5. Discussion

Weed menace is a serious threat to faba bean production, and the identification of herbicide-tolerant varieties is one of the most effective methods for weed control. The results obtained from the present field and greenhouse studies demonstrated how the post-emergence application of metribuzin or imazethapyr negatively affects faba bean plants. Herbicide application affected the crop phenology by delaying flowering and maturity. Although the delayed flowering helps plant escape the risk of frost in regions like Western Australia, there might be a potential yield penalty as the plants run out of moisture before it can fill its grain^{43,44}. In addition, herbicide application also affected biological and grain yields of faba bean by reducing plant height, green canopy cover, and grain yield components and by increasing the number of branches. Many studies^{30,42,45,46,47,48} reported significant reduction in plant height, grain yield and yield components while studying the effect of post-emergence herbicide application on faba bean, lentil and chickpea. On the other hand, Wall⁴⁹ and Sajja et al.⁵⁰, reported an increase in the number of branches of treated plants. The observed damage after metribuzin and imazethapyr treatments is the consequence of the growth inhibition caused by both herbicides. Metribuzin hampers photosynthesis activity by inhibiting the photosynthetic electron flow^{51,52} and imazethapyr inhibits acetolactate synthase (ALS)⁵³, the first common enzyme in the biosynthesis of the branched-chain amino acids⁵⁴ causing the death of meristematic cells. On the other hand, significant increase in the number of branches in herbicide treated plants could be caused by the plant recovery which occurs at the lateral meristem in dicots resulting in the development of new branches. The genotypic variation observed in the herbicide damage scores (HDS) highlights the difference in the reaction of each genotype toward post emergence herbicide application in faba bean. This observation was expected as the evaluated genotypes are genetically diverse²². The differences observed between the first (HDS1) and the second (HDS2) scores were due to the recovery or deterioration of the plants one month after herbicide treatment. The recovery might result from the metabolism of the herbicides into inactive compounds⁵⁵. Therefore, the observed differences in the genotype ability to recover might be due to differential rate of metabolic degradation for imazethapyr treatment⁴⁷ and to differential disruption of electron transfer for metribuzin treatment.

Population structure analysis revealed two major ancestral populations for the germplasm which is compatible with the original germplasm of 995 genotypes genotyped with 20 microsatellite markers, from which this population was selected²². As expected, MT-GWAS analysis exposed higher detection power compared to ST-GWAS analysis due to the larger datapoint fitted in the

model which is equivalent to increasing the population size²¹. This was revealed by the larger number of highly significant as well as suggestive association per trait detected (Table 3, Supplementary Table S3). Another advantage is the ability to detect QTL with stable effect across different environments or treatments which should have higher potential to improve the efficiency of marker assisted selection in diverse environments^{56,57}.

To the best of our knowledge, the present study is the first GWAS for herbicide tolerance in faba bean and the first for all phenotyped traits under the control treatment with no herbicide application. Thus, most of the QTL detected in the present study seem novel and have not been reported before. Very limited studies used SNP data on biparental or multi-parental faba bean populations^{15,26,58} but none aimed to dissect quantitative traits in natural diverse populations. Sallam and Martsch⁵⁸ associated 156 SNPs with frost tolerance in a population derived from 11 parental lines, while Ali et al.⁵⁹ used the same population to detect loci associated with freezing and drought tolerance using 175 SNPs and AFLP markers. The identification of QTL through GWAS in faba bean is complex due to the large undecoded genome and highly repetitive sequences. These issues have delayed the progress made towards the development of genomic resources and marker assisted selection in faba bean breeding programs⁶⁰.

Identification of key genes, mechanisms and functional markers is essential to develop herbicide tolerant faba beans. The associations between some genes identified in this study and herbicide tolerance have been reported previously in different crops. Acidic endochitinase and malate dehydrogenase which were found to be associated with the reduction indexes of DFLR and GCC were among the proteins affected by the application of sulfonylurea herbicide in soybeans⁶¹. Sulfonylurea herbicides and imazethapyr have similar mode of action; both herbicides block the biosynthesis of the branched-chain amino acids^{54,62}. The two protein kinase LRR receptor-like serine/threonine-protein kinase RCH1 and probable serine/threonine-protein kinase NAK which were found to be associated with the reduction index of PLHT and GYPLT in the present study are generally considered key regulators of plant architecture and growth behavior, and the expansion of these proteins during plant evolution has also been correlated with the specific adaptations of the species in defense and stress responses⁶³. Their direct involvement in abiotic stress resistance (drought, heat, cold, salinity) has also been demonstrated in different studies^{64,65,66}. Burns et al.⁶⁷ concluded that herbicide stress is perceived similarly to other abiotic stresses and reported modification in the level of the protein kinase gene family in the multiple herbicide resistant *Avena fatua*.

The MYB-related protein P-like which was found associated with DFLR is involved in herbicide tolerance belongs to the *MYB* gene family that comprises one of the richest groups of transcription factors in plants. Members of this family have a well-established role in abiotic stress responses^{68,69}. Bhoite et al.⁷⁰ found also that the transcription factors MYB were significantly expressed under metribuzin stress. The photosystem I (PS I) core protein PsaA that is found in the present study to be associated with the reduction index of NPPLT is a subunit membrane protein complex involved in photosynthesis. PS I and PS II drive the light reaction of photosynthesis. The first stage of the light reaction occurs in PS II whereas the final stage of the light reaction occurs in PS I⁷¹. The metribuzin applied to faba bean plants in this study inhibits PS II by disrupting electron transfer through binding to the D1 protein of the photosystem II complex in chloroplast thylakoid membranes⁵¹. This mode of action explains the involvement of the PS I in the reaction toward herbicide application especially that PS II comes first in the path of the electron flow followed by PS I.

The described mechanism of action of the annotated genes suggests that DFLR_RI and GCC_RI are associated with tolerance to imazethapyr while DFLR and NPPLT_RI are associated with tolerance to metribuzin, and GYPLT and PLHT_RI are associated with tolerance to both herbicides.

6. Conclusions

Weeds represent a major problem to faba bean crop which limits its expansion in many production regions. By excluding faba bean and other legume from the cropping system, cereal monoculture will continue to deplete the soil, lowering its quality and indirectly reducing yield and quality of the produce. Herbicide tolerant faba bean lines could be a game changer in the reintegration of faba bean in modern cropping systems as it contributes to the reduction of production cost by avoiding excessive use of manual weeding. Considering the many advantages of herbicide tolerance in faba bean, it is imperative to breed elite cultivars that features this trait. However, field selection is very costly and time consuming. The integration of genomic selection and marker assisted selection will improve selection accuracy, increase the selection intensity and shorten the breeding cycle when selecting at early generations. In the present study, we identified genomic regions associated with tolerance to imazethapyr and metribuzin herbicides as highly significant associations between SNPs markers and phenological and yield traits related to herbicide tolerance were detected using multi-trait association. These markers will be useful for improving the efficiency of faba bean programs and represent important steps towards the selections for herbicide tolerance.

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8. Supplementary Materials

Supplementary Table S1. Details of the traits recorded at each experiment; traits are coded as the environment, followed by the trait, the treatment and “RI” if the number describe a reduction index.

Environment	Trait					
	Terbol 2016 Imazethapyr	Terbol 2016 Metribuzin	Terbol 2016 Control	Marchonch 2015 Imazethapyr	Marchonch 2015 Metribuzin	Marchonch 2015 Control
HDS2	TR16HDS2I	TR16HDS2M	NA	NA	MR15HDS2M	MR15HDS2I
HDS1	TR16HDS1I	TR16HDS1M	NA	NA	NA	NA
GCC_RI	NA	NA	NA	NA	NA	NA
GCC	NA	NA	NA	NA	NA	NA
NB+PLT_RI	NA	NA	NA	NA	NA	NA
NB+PLT	NA	NA	NA	NA	NA	NA
GYPLT_RI	TR16GYPLT_RI	TR16GYPLTM_RI	NA	NA	NA	NA
GYPLT	TR16GYPLTI	TR16GYPLTM	TR16GYPLTC	MR15GYPLTI	MR15GYPLTM	MR15GYPLTC
NSPLT_RI	TR16NSPLT_RI	TR16NSPLTM_RI	NA	NA	NA	NA
NSPLT	TR16NSPLTI	TR16NSPLTM	TR16NSPLTC	NA	NA	NA
PLHT_RI	TR16PTHTI_RI	TR16PTHTM_RI	NA	RMRI15PLTHTI_RI	RMRI15PLTHTM_RI	NA
PLHT	TR16PTHTI	TR16PTHTM	TR16PTHTC	RMRI15PLTHTI	RMRI15PLTHTM	TR19PLHTI
NPPLT_RI	TR16NPPLT_RI	TR16NPPLTM_RI	NA	NA	NA	NA
NPPLT	TR16NPPLTI	TR16NPPLTM	TR16NPPLTC	NA	NA	NA
DMAT_RI	TR16DMATI_RI	TR16DMATM_RI	NA	NA	NA	NA
DMAT	TR16DMATI	TR16DMATM	TR16DMATC	NA	NA	MR15DMATC
DFLR_RI	TR16DFLR_RI	TR16DFLRM_RI	NA	NA	NA	NA
DFLR	TR16DFLRI	TR16DFLRM	TR16DFLRC	NA	NA	MR15DFLRC

TR19HDS21	TR19HDS2M	NA	TRS18HDS2M	NA
NA	NA	NA	TRS18HDS1M	NA
TR19GCC1_RI	TR19GCCM_RI	NA	TRS18GCCM_RI	NA
TR19GCC1	TR19GCCM	TR19GCC	TRS18GCCM	TRS18GCC
TR19NBpPLTL_RI	TR19NBpPLTM _{DI}	NA	NA	NA
TR19NBpPLTL	TR19NBpPLTM	TR19NBpPLTC	NA	NA
NA	NA	NA	NA	NA
NA	NA	NA	NA	NA
TR19NSPLTL_RI	TR19NSPLTM _R	NA	NA	NA
TR19NSPLTL	TR19NSPLTM	TR19NSPLTC	NA	NA
TR19PLHTM_RI	TR19PLHTC_RI	NA	TRS18PLHTM_RI	NA
TR19PLHTM	TR19PLHTC	NA	TRS18PLHTM	TRS18PLHTC
TR19NPPLTL_RI	TR19NPPLTM _R	NA	NA	NA
TR19NPPLTL	TR19NPPLTM	TR19NPPLTC	NA	NA
TR19DMATL_RI	TR19DMATM _R	NA	NA	NA
TR19DMATL	TR19DMATM	TR19DMATC	NA	NA
TR19DFLRL_RI	TR19DFLRM_RI	NA	NA	NA
TR19DFLRL	TR19DFLRM	TR19DFLRC	NA	NA
Terbol 2019 Imazethapyr	Terbol 2019 Metribuzin	Terbol 2019 Control	Terbol Summer 2018 Metribuzin	Terbol Summer 2018 Control

DFLR days to flowering, DMAT days to maturity, NPPLT number of pods per plant, PLHT plant height, NSPLT number of seeds per plant, GYPLT grain yield per plant, NBrPLT number of branches per plant, GCC green canopy cover, HDS1 herbicide damage HDS2 recorded at the flowering stage, HDS2 herbicide damage HDS2 recorded at the pod development stage, RI Reduction index, NA not applicable.

Supplementary Table S2. Description of the damages observed in the treated plants for each herbicide damage score (HDS)

Herbicide damage score (HDS)	Description
1	No damage observed
	Normal phytosanitary status
	Normal and very good vegetative growth
2	Very light damage observed
	Very few leaf burnings
	Very good phytosanitary status
3	A clear moderate damage observed
	Stunting in growth with high yellowing
	Necrosis on leaves
4	A high damage was observed and death of <50% of plants
	Severe yellowing, leaf and stem burning with high deformations
	Very weak vegetative growth and stunted plants
5	Severe damage and death of >50% of plants
	High deformations and burnings
	High reduction of plant's biomass
	Overall yellowing was detected

Supplementary Table S3. SNP-trait associations revealed by the ST-GWAS analysis. Underscored SNPs represents the highly significant associations, while SNPs in italic represents the SNPs associated with multiple traits

Trait	SNP	allele1	allele0	MAF	Effect	P	-Log10
TR16_DFLRI_RI	<u>SNODE_5725_31</u>	C	T	0.41	5.66	4.3E-11	10.4
TR16_DFLRI_RI	<u>SCONTIG127798_41</u>	C	T	0.07	-2.60	5.0E-11	10.3
TR16_DFLRM_RI	<u><i>SNODE_23759_68</i></u>	A	G	0.10	6.67	2.1E-07	6.7
MR15_PLTHTI	<u>SNODE_106460_36</u>	T	C	0.05	18.29	4.3E-07	6.4
TRS18_HDS1M	<u>SNODE_13467_45</u>	A	G	0.19	0.70	1.1E-06	6.0
MR15_GYPLTI	<u>SNODE_4555_43</u>	T	A	0.10	12.18	1.5E-06	5.8
TR16_DFLRI	<u><i>SNODE_7114_58</i></u>	C	T	0.11	6.71	2.5E-06	5.6
TR19_NBrPLTM_RI	<u>SNODE_173108_18</u>	A	G	0.06	65.35	3.0E-06	5.5
TRS18_GCCC	<u>SNODE_12390_16</u>	C	T	0.21	1.27	3.0E-06	5.5
TR16_DFLRM_RI	<u><i>SNODE_4187_38</i></u>	G	A	0.05	7.81	3.3E-06	5.5
TR16_NSPLTM	SCONTIG38056_40	C	T	0.07	9.44	5.9E-06	5.2
TR19_NPPLTI	SCONTIG16487_99	T	C	0.07	5.53	6.1E-06	5.2
TR16_DMATI	SNODE_76542_45	T	G	0.05	-3.24	7.0E-06	5.2
MR15_PLTHTM_RI	<u><i>SNODE_14298_44</i></u>	A	G	0.06	63.37	7.1E-06	5.1
TR16_GYPLTI	<u><i>SNODE_77186_51</i></u>	T	A	0.10	7.97	7.2E-06	5.1
MR15_PLTHTI_RI	SCONTIG65312_44	T	C	0.05	89.46	9.1E-06	5.0
MR15_PLTHTI_RI	SNODE_783_18	G	A	0.06	83.04	1.0E-05	5.0
TR19_NBrPLTM_RI	SNODE_2942_50	C	T	0.06	61.37	1.0E-05	5.0
TR19_NBrPLTI_RI	SNODE_103_72	T	C	0.25	27.28	1.1E-05	5.0
TR16_DFLRI_RI	SCONTIG5600_39	C	T	0.06	7.01	1.2E-05	4.9
TR16_DFLRM	<u><i>SNODE_7114_58</i></u>	C	T	0.11	6.69	1.2E-05	4.9
TR19_GCCC	SNODE_46550_53	C	A	0.41	5.17	1.5E-05	4.8
TRS18_HDS2M	SNODE_132611_47	T	G	0.06	1.48	1.5E-05	4.8
TR16_NPPLTTI_RI	SNODE_522694_66	T	C	0.07	53.48	1.6E-05	4.8
TR19_GCCM_RI	SNODE_18326_71	G	A	0.37	14.61	1.6E-05	4.8
MR15_GYPLTM	SNODE_32018_65	A	G	0.20	6.33	1.6E-05	4.8
TR19_GYPLTI	SNODE_4363_81	C	T	0.34	4.00	1.7E-05	4.8
TRS18_HDS1M	SCONTIG65425_78	T	C	0.22	0.52	1.7E-05	4.8
TR16_DMATI	SNODE_5210_41	G	A	0.24	-1.31	1.8E-05	4.7
TR16_DMATI	SNODE_5352_33	C	T	0.43	-1.23	1.9E-05	4.7
MR15_PLTHTI_RI	<u><i>SNODE_303749_26</i></u>	G	C	0.09	55.58	1.9E-05	4.7
TR16_PLHTI_RI	<u><i>SNODE_168698_34</i></u>	G	A	0.31	-7.68	2.0E-05	4.7
TR19_DMATM	SNODE_1938_61	T	G	0.10	0.91	2.1E-05	4.7
TR16_DMATC	<u><i>SNODE_7114_58</i></u>	C	T	0.11	1.67	2.2E-05	4.7
TR19_DMATC	SNODE_176062_39	G	T	0.39	0.66	2.2E-05	4.7
TR16_PLHTM	SNODE_1327_40	G	A	0.26	-4.92	2.2E-05	4.7
MR15_PLTHTI_RI	SCONTIG16540_74	G	A	0.06	78.04	2.3E-05	4.6
MR15_GYPLTI	SNODE_49416_94	A	G	0.07	12.46	2.4E-05	4.6

TR19_DMATI	SCONTIG93219_127	A	C	0.05	2.09	2.5E-05	4.6
TR19_NPPLTC	SNODE_159494_68	G	A	0.07	6.96	2.7E-05	4.6
MR15_PLHTTI	SNODE_4025_42	T	G	0.06	16.12	2.7E-05	4.6
MR15_PLHTTI_RI	SNODE_11304_26	G	A	0.13	44.41	2.8E-05	4.6
MR15_PLHTTI_RI	<i>SNODE_14298_44</i>	A	G	0.06	58.04	2.8E-05	4.6
TR19_NSPI	SNODE_99859_41	C	A	0.05	11.79	2.8E-05	4.5
MR15_GYPLTM	SCONTIG8042_30	T	G	0.14	9.13	2.9E-05	4.5
TR16_DFLRI	<i>SNODE_27970_52</i>	C	G	0.17	4.79	3.0E-05	4.5
TRS18_PLHT2C	SNODE_8117_82	T	C	0.41	-3.63	3.0E-05	4.5
TR16_DMATI	SNODE_5497_184	C	T	0.07	-2.62	3.1E-05	4.5
TR16_NPPLTTM_RI	SNODE_13896_44	T	G	0.21	33.37	3.1E-05	4.5
MR15_GYPLTM	SCONTIG124093_52	T	A	0.06	14.06	3.1E-05	4.5
MR15_PLHTTM_RI	SNODE_154943_14	T	C	0.08	52.28	3.4E-05	4.5
MR15_GYPLTI	SNODE_113699_29	T	C	0.17	8.18	3.5E-05	4.5
TR19_ScoreI	SNODE_6947_50	A	G	0.22	0.15	3.6E-05	4.4
TR16_GYPLTM_RI	<i>SNODE_22383_32</i>	T	C	0.14	23.39	3.6E-05	4.4
TR16_DFLRI_RI	SNODE_12919_43	A	T	0.11	3.97	3.6E-05	4.4
TR16_DMATM	SCONTIG38602_68	G	A	0.06	-2.68	3.7E-05	4.4
TR19_DFLRM	SNODE_73156_58	C	T	0.19	4.17	3.7E-05	4.4
TR19_NPPLTI	SNODE_13244_37	C	T	0.08	4.84	3.7E-05	4.4
TR16_GYPLTM	<i>SNODE_3696_16</i>	G	A	0.10	62.71	3.9E-05	4.4
TRS18_GCCC	SNODE_11304_24	A	T	0.31	-0.89	4.1E-05	4.4
TR19_ScoreI	SCONTIG57859_65	G	A	0.07	-0.31	4.2E-05	4.4
TR19_DMATI	<i>SNODE_239220_75</i>	G	C	0.41	1.03	4.3E-05	4.4
MR15_PLHTTM_RI	<i>SNODE_303749_26</i>	G	C	0.09	54.27	4.3E-05	4.4
TR19_NBrPLTM_RI	SNODE_144193_69	C	A	0.46	-66.48	4.4E-05	4.4
MR15_GYPLTI	SCONTIG90061_39	A	C	0.06	13.39	4.4E-05	4.4
TR16_GYPLTM	<i>SNODE_3696_16</i>	G	A	0.10	5.23	4.5E-05	4.3
TRS18_PLHT2M	SNODE_50475_21	A	G	0.23	3.85	4.5E-05	4.3
TR16_DFLRM	<i>SNODE_27970_52</i>	C	G	0.16	5.02	4.6E-05	4.3
TR16_DFLRM_RI	SNODE_1051_18	C	T	0.08	5.93	4.8E-05	4.3
TR16_PLHTC	SCONTIG124142_38	A	G	0.11	-7.42	5.0E-05	4.3
TR16_HDS1I	SCONTIG124448_24	G	A	0.47	0.91	5.0E-05	4.3
MR15_PLHTTI_RI	SCONTIG86606_73	G	A	0.07	75.29	5.1E-05	4.3
TR19_NBrPLTC	SCONTIG97891_72	A	G	0.40	0.64	5.3E-05	4.3
TR19_GCCI_RI	SNODE_8714_56	G	A	0.12	26.15	5.4E-05	4.3
MR15_GYPLTC	SCONTIG107603_117	G	A	0.33	8.52	5.5E-05	4.3
TR16_DFLRM	<i>SNODE_23759_68</i>	A	G	0.10	8.39	5.6E-05	4.3
TR16_DFLRC	<i>SNODE_7114_58</i>	C	T	0.11	3.49	5.6E-05	4.3
TR16_NSPLTI	SNODE_27984_41	T	C	0.09	-6.51	5.6E-05	4.2
TRS18_GCCM_RI	SNODE_6438_25	T	A	0.28	23.95	5.7E-05	4.2
TR19_NBrPLTC	SNODE_1799_57	A	T	0.16	0.52	5.7E-05	4.2

TR16_GYPLTM_RI	SNODE_22383_32	T	C	0.14	21.58	5.9E-05	4.2
MR15_PLTHTI_RI	SNODE_6940_56	C	T	0.13	42.12	5.9E-05	4.2
TR19_NSPM	SNODE_784518_64	C	T	0.43	-4.05	6.0E-05	4.2
TR16_DFLRM_RI	SCONTIG20800_23	G	A	0.06	6.56	6.0E-05	4.2
MR15_PLTHTI_RI	SNODE_2199_68	G	A	0.07	68.70	6.2E-05	4.2
TR16_PLHTI	SNODE_168698_34	G	A	0.31	-5.44	6.2E-05	4.2
TR16_NPPLTTM_RI	SNODE_55885_28	C	T	0.09	47.66	6.3E-05	4.2
TR19_DMATI_RI	SNODE_13235_37	C	A	0.25	-0.43	6.3E-05	4.2
TR16_PLHTM_RI	SCONTIG99169_68	C	T	0.05	-12.13	6.6E-05	4.2
MR15_GYPLTI	SCONTIG46666_46	A	T	0.07	12.22	6.7E-05	4.2
TR16_DFLRI_RI	SNODE_4187_38	G	A	0.05	6.82	6.8E-05	4.2
MR15_PLTHTI_RI	SNODE_34274_45	A	G	0.11	46.06	7.0E-05	4.2
TR16_NPPLTTI_RI	SNODE_503024_18	T	A	0.40	23.17	7.3E-05	4.1
TR16_NSPLTC	SNODE_125479_23	A	T	0.28	5.86	7.3E-05	4.1
TR16_DFLRC	SNODE_9493_68	T	C	0.06	4.19	7.3E-05	4.1
TR16_DFLRM_RI	SNODE_8481_51	T	C	0.17	3.50	7.4E-05	4.1
TR19_NPPLTI_RI	SCONTIG63866_41	T	C	0.34	-14.64	7.5E-05	4.1
TR19_DFLRI_RI	SNODE_84004_43	T	G	0.23	1.14	7.7E-05	4.1
TRS18_HDS2M	SNODE_7070_46	T	G	0.09	0.91	7.9E-05	4.1
TR19_GYPLTC	SNODE_35312_45	A	T	0.38	5.77	7.9E-05	4.1
TR19_NPPLTI	SCONTIG106679_64	C	T	0.07	4.70	8.1E-05	4.1
TR19_NPPLTM	SNODE_134625_31	C	T	0.44	-2.15	8.1E-05	4.1
TR19_NSPI_RI	SNODE_2075_83	C	T	0.18	15.10	8.3E-05	4.1
TR19_DMATI	SNODE_3763_57	C	T	0.21	-0.81	8.4E-05	4.1
MR15_PLTHTM	SNODE_53396_78	G	T	0.23	7.29	8.4E-05	4.1
MR15_GYPLTC	SNODE_139623_44	A	T	0.36	12.78	8.5E-05	4.1
TR19_GCCM_RI	SCONTIG50118_65	G	A	0.08	-29.38	8.5E-05	4.1
TR16_DMATM_RI	SNODE_6679_53	T	A	0.48	0.71	8.9E-05	4.1
TR16_NPPLTTI_RI	SNODE_239220_75	G	C	0.41	30.20	8.9E-05	4.1
TR19_GYPLTM	SNODE_12261_51	C	T	0.06	-9.39	8.9E-05	4.0
TR19_GCCI_RI	SNODE_10934_67	A	G	0.35	-18.74	9.0E-05	4.0
MR15_PLTHTI_RI	SNODE_106637_40	C	T	0.07	73.72	9.1E-05	4.0
TR16_DFLRC	SNODE_27970_52	C	G	0.16	2.75	9.3E-05	4.0
TR19_GYPLTM_RI	SCONTIG7685_19	A	G	0.06	23.11	9.4E-05	4.0
TR16_DFLRI	SNODE_162178_22	A	G	0.07	8.88	9.5E-05	4.0
MR15_GYPLTM	SCONTIG61848_71	G	A	0.07	10.97	9.7E-05	4.0
TR19_DFLRM_RI	SNODE_7114_58	C	T	0.12	1.39	9.7E-05	4.0
TR16_DMATC	SNODE_12561_61	A	C	0.08	2.38	9.8E-05	4.0

Supplementary Table S4. Gene annotation information of some of the highly significant NPSs-traits associations.

SNP	Gene	Associated traits
CONTIG127798	acidic endochitinase	DFLR_RI; GCC_RI
NODE 14298 length 69 cov 191.202896	LRR receptor-like serine/threonine-protein kinase RCH1	PLHT_RI
NODE 3696 length 73 cov 344.041107	Probable serine/threonine-protein kinase NAK	GYPLT
NODE 4187 length 70 cov 94.728569	malate dehydrogenase	DFLR_RI
NODE 559376 length 95 cov 1.252632	photosystem I core protein PsaA	NPPLT_RI
NODE 7114 length 70 cov 20.785715	MYB-related protein P-like	DFLR

CHAPTER V

General Conclusions

- (i) Postemergence application of both imazethapyr and metribuzin causes delay in flowering and maturity and reduction in plant height, yield components, and grain yield (Chapter II).
- (ii) The reduction index of grain yield (RI_{GY}) is considered the most effective criteria for selecting faba bean for herbicide tolerance (Chapter II).
- (iii) The harmful effects of herbicides intensify with the increase in the herbicide doses (Chapter II).
- (iv) Genetic variability for herbicide tolerance is available in faba bean germplasm and tolerance to metribuzin and imazethapyr was confirmed in eight faba bean accessions (VF335, FB2528, ILB132194, B2574, FB1482, IG12659, ILB1814, Flip 86-98FB) with no significant reduction in grain yield (Chapter II).
- (v) Plant height and grain yield stability should be assessed based on a combination of stability parameters (Chapter III).
- (vi) IG12983 is a superior genotype as it showed a good and stable performance for plant height and grain yield across locations, seasons, and herbicide treatments (Chapter III).
- (vii) Some accessions namely IG13945, IG13906, IG106453, FB2648, and FB1216 had specific adaptation to at least one of the defined mega environments but not to others (Chapter III).
- (viii) The environments that are less likely to experience drought are recommended for herbicide screening to avoid the confounding effect of herbicides and drought (Chapter III).
- (ix) The screened faba bean population is structured of two ancestral subpopulations.
- (x) Multi-trait (MT) Genome Wide Association Studies (GWAS) identified 14 highly significant associations between SNPs markers and phenological and yield traits related to herbicide tolerance (Chapter IV).
- (xi) SNPs from acidic endochitinase, LRR receptor-like serine/threonine-protein kinase RCH1, probable serine/threonine-protein kinase NAK, malate dehydrogenase, photosystem I core protein PsaA and MYB-related protein P-like were significantly associated with herbicide tolerance traits (Chapter IV).