

## ORIGINAL RESEARCH ARTICLE

## Plant Genetic Resources

# Identification of tolerance to metribuzin and imazethapyr herbicides in faba bean

Lynn Abou-Khater<sup>1</sup>  | Fouad Maalouf<sup>1</sup>  | Somanagouda B. Patil<sup>2</sup> | Rind Balech<sup>1</sup> | Diana Nacouzi<sup>3</sup> | Diego Rubiales<sup>4</sup>  | Shiv Kumar<sup>2</sup> 

<sup>1</sup> International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol, Lebanon

<sup>2</sup> ICARDA, Rabat, Morocco

<sup>3</sup> Faculty of Agriculture, Lebanese University, Beirut, Lebanon

<sup>4</sup> Institute for Sustainable Agriculture, CSIC, Córdoba 14004, Spain

## Correspondence

Lynn Abou Khater, International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol, Lebanon

Email: [lynnaboukhater@hotmail.com](mailto:lynnaboukhater@hotmail.com)

Fouad Maalouf, International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol, Lebanon

Email: [f.maalouf@cgiar.org](mailto:f.maalouf@cgiar.org)

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## 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<sup>-1</sup> and imazethapyr at 75 g a.i. ha<sup>-1</sup> 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<sup>-1</sup> and from 75 to 150 g a.i. ha<sup>-1</sup>. Tolerance to metribuzin and imazethapyr in eight faba bean accessions was confirmed with no significant reduction in grain yield.

**Abbreviations:** ALS, acetolactate synthase; BRPLT, number of branches per plant; DFLR, days to flowering; DMAT, days to maturity; GYPLT, grain yield per plant; HDS, herbicide damage score; PLHT, plant height; PNPLT, number of pods per plant; RI, reduction index; RIGY, reduction index of grain yield per plant; RIHT, reduction index of plant height; SNPLT, number of seeds per plant; WANA, western Asia and northern Africa.

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## 1 | 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](http://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](http://www.faostat.fao.org)). This is because of various biotic and abiotic stresses and agricultural practices such as the limitation of efficient post emergence 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<sup>-1</sup>. 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 rota-

### Core Ideas

- Integration of faba bean in the cropping system increases its sustainability & soil fertility.
- The sources for herbicide tolerance will be combined with other traits to develop new cultivars.
- The identified lines reduce the cost of the production by limiting the laborious hand weeding.
- The identified new sources of herbicide tolerance in faba bean will be the first reported.
- Scope toward the development of postemergence herbicide tolerant faba bean cultivars.

tions, soil solarization, and herbicide applications, are recommended for effective weed control in faba bean (Burnside 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 (Garcia 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—leucine, isoleucine, 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; Tan 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

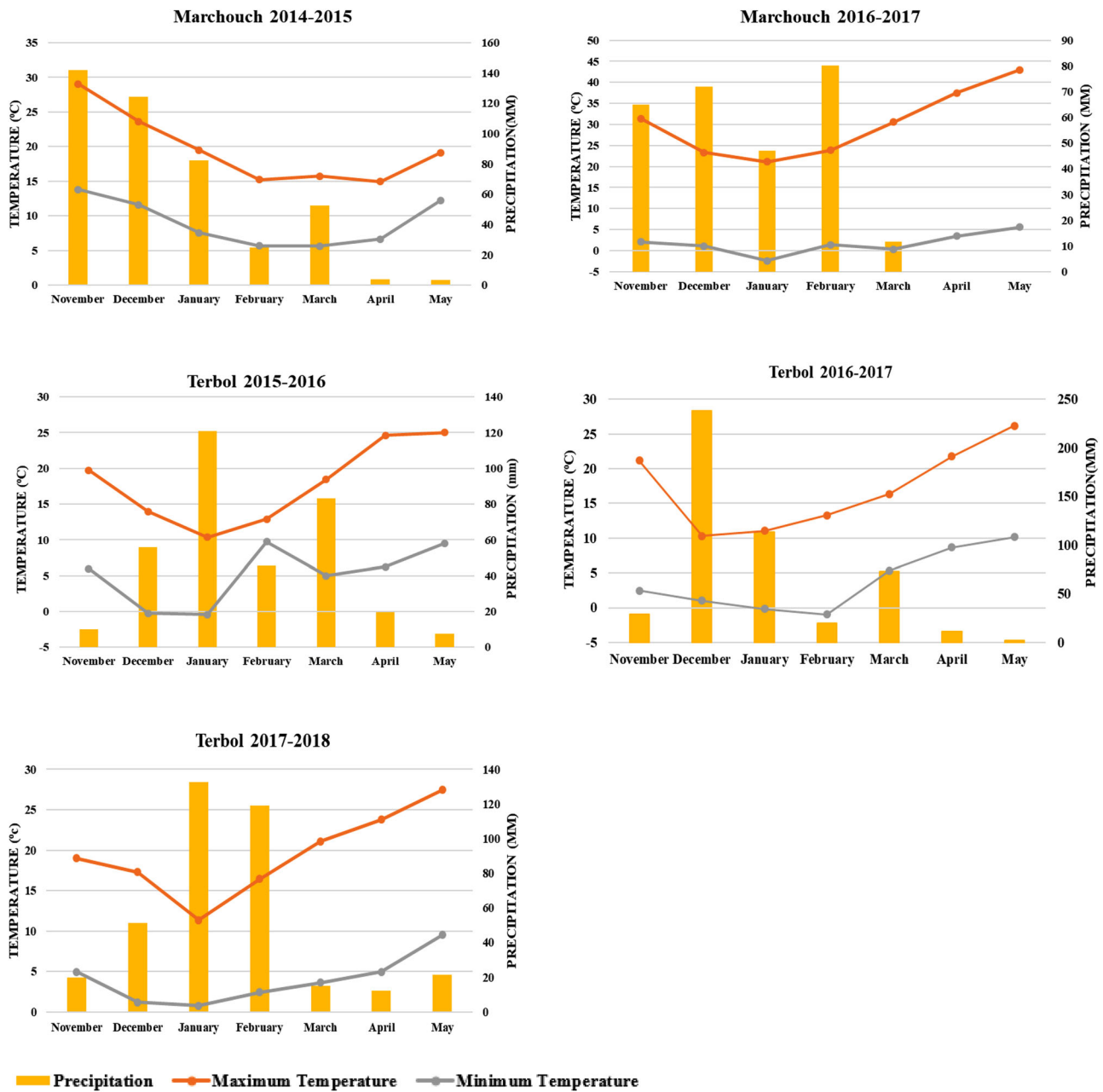


FIGURE 1 Maximum temperature (°C), minimum temperature (°C), and precipitation (mm) at Marchouch and Terbol during different planting seasons

[*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.

## 2 | MATERIALS AND METHODS

### 2.1 | 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.

## 2.2 | 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<sup>-1</sup> and spraying lambda-cyhalothrin at 40 g a.i. ha<sup>-1</sup> to control sitona, imidacloprid at 160 g a.i. ha<sup>-1</sup> to control aphids, and a combination of azoxystrobin and difenoconazole at 72.8 and 45.6 g a.i. ha<sup>-1</sup>, respectively, to control foliar diseases. Weeds were controlled by pre-emergence application of pendimethalin at 1,200 g a.i. ha<sup>-1</sup> followed by manual weeding to avoid weed competition. The major weeds found in our fields at both locations were bean broomrape (*Orobancha crenata* Forssk.), dodder (*Cuscuta campestris* Yunck.), rapeseed (*Brassica napus* L.), bindweed (*Convolvulus arvensis* L.), and narrow-leaved weeds.

## 2.3 | 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<sup>-1</sup> (T1) and imazethapyr at 75 g a.i. ha<sup>-1</sup> (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.

## 2.4 | 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.

## 2.5 | 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<sup>-1</sup>, T3 = 375 g a.i. ha<sup>-1</sup>, and T4 = 500 g a.i. ha<sup>-1</sup>) and imazethapyr (T2 = 75 g a.i. ha<sup>-1</sup>, T5 = 112.5 g a.i. ha<sup>-1</sup>, and T6 = 150 g a.i. ha<sup>-1</sup>) dosages along with control

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, VF510, VF512, VF513, VF522	Sudan	IG13945	
	Ethiopia	IG11742, IG11908, IG14026, VF268	Switzerland	FB1682, FB1709	
	France	FB310, FB1564, FB2077, FB2509, FB2528, FB2583	Syrian Arab Republic	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 Arab Republic	IG70622	
Germany		FB1512	Tunisia	VF544, IG12983	
ICARDA <sup>a</sup>		IG104985, IG105789, IG105844, IG103102, IG104421, IG104526, IG104821	Turkey	IG11388	
Iraq		IG11232	United Kingdom	FB1213	
Morocco		IG13764			

(Continues)



TABLE 1 (Continued)

Subspecies	Country	Accessions	Country	Accessions
<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 Arab Republic	IG13008, IG13958, IG72498
	ICARDA <sup>a</sup>	IG103043	Ukraine	IG130407
	Italy	IG13231	Unknown	VF260
<i>Vicia faba</i> var. <i>paucijuga</i>	ICARDA <sup>a</sup>	IG104039	ICARDA <sup>a</sup>	IG104082
	Russia	VF335	Czech Republic	VF301
	Nepal	IG115213	ICARDA	IG106984
	China	IG126166	United Kingdom	VF626, VF810

<sup>a</sup>Breeding lines.

(C) were evaluated in an unbalanced block design with three replications.

## 2.6 | Observations recorded

Herbicide damage score (HDS) was recorded twice as a preliminary observation using a 1-to-5 scale 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:

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

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.

## 2.7 | 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

**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

(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.

### 3 | RESULTS

#### 3.1 | Herbicide damage score

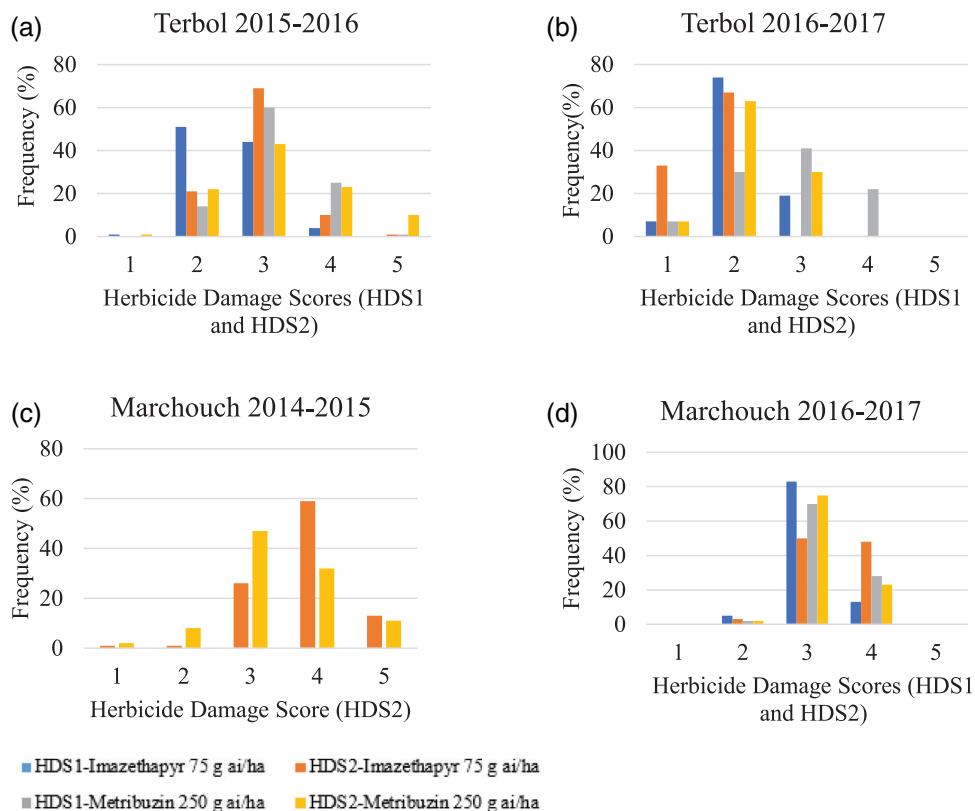
Faba bean accessions treated with metribuzin and imazethapyr were given a 1-to-5 score (Table 2) at the first HDS (HDS1) stage, indicating a wide range of genotypic variation among the tested accessions across locations and seasons. Most of the accessions treated with metribuzin

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



**FIGURE 2** Distribution of faba bean accessions for herbicide damage scores (HDS1 and HDS2) under metribuzin at 250 g a.i. ha<sup>-1</sup> and imazethapyr at 75 g a.i. ha<sup>-1</sup> in preliminary (a and c) and validation screenings (b and d)

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 wk 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<sup>-1</sup> resulted in identification of one accession (FB2583) showing no damage (HDS1 = 1). Increasing metribuzin dose to 375 and 500 g a.i. ha<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup>. The HDS2 score showed no remarkable change in imazethapyr treatments at 75 and 112.5 g a.i. ha<sup>-1</sup>, but in imazethapyr at 150 g a.i. ha<sup>-1</sup>, 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.



### 3.2 | 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<sup>-1</sup> of imazethapyr and 250 g a.i. ha<sup>-1</sup> 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<sup>-1</sup> (T4) as compared with 90–109 d with metribuzin at 250 g a.i. ha<sup>-1</sup>. 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.

### 3.3 | 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 acces-

sions 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<sup>-1</sup>) and 53.4 cm under T6 (imazethapyr at 150 g a.i. ha<sup>-1</sup>). 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).

### 3.4 | 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<sup>-1</sup> (T1) or with imazethapyr at 75 g a.i. ha<sup>-1</sup> (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<sup>-1</sup>) and imazethapyr (112.5 and 150 g a.i. ha<sup>-1</sup>) 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<sup>-1</sup> and 9.9 with metribuzin at 500 g a.i. ha<sup>-1</sup> (Table 4). Similar results were observed for metribuzin treatments for SNPLT.

**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 × T interaction, expressed as *P* value

Step	Site, season	df	Trait <sup>a</sup>																			
			DFLR	DMAT	PLHT	RI <sub>HT</sub>	PNPLT	SNPLT	GYPLT	RI <sub>GY</sub>	HSW	BRPLT										
Preliminary screening	Marchouch, 2014–2015																					
	A	132	.046	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.054	ND <sup>b</sup>	ND	ND	ND	ND	ND	ND	
	T	2	ND	ND	<.001	<.002	.013	.013	.013	.013	.013	.013	.013	.093	ND	ND	ND	ND	ND	ND	ND	
	A × T	164	ND	ND	.057	.932	.279	.279	.279	.279	.279	.279	.279	.481	ND	ND	ND	ND	ND	ND	ND	
	Terbol, 2015–2016																					
	A	133	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.050	ND	ND	ND	ND	ND	ND	ND
T	2	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.004	ND	ND	ND	ND	ND	ND	ND	
A × T	266	<.001	.258	<.001	<.001	.140	.140	.140	.140	.140	.140	.140	.140	.051	ND	ND	ND	ND	ND	ND	ND	
Validation trial	Marchouch, 2016–2017																					
	A	39	.306	.008	<.001	.559	ND	ND	ND	ND	ND	ND	ND	.048	ND	ND	ND	ND	ND	ND	ND	
	T	2	<.001	<.001	<.001	.380	ND	ND	ND	ND	ND	ND	ND	.499	ND	ND	ND	ND	ND	ND	ND	
	A × T	78	<.001	.992	.007	.384	ND	ND	ND	ND	ND	ND	ND	.010	ND	ND	ND	ND	ND	ND	ND	
	Terbol, 2016–2017																					
	A	26	<.001	<.001	<.001	.036	<.001	<.001	<.001	<.001	.014	.014	.014	.014	.637	ND	ND	ND	ND	ND	ND	ND
T	2	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.041	.041	.041	.041	<.001	ND	ND	ND	ND	ND	ND	ND	
A × T	52	.901	.332	.028	.001	.551	.551	.551	.551	.345	.345	.345	.345	.881	ND	ND	ND	ND	ND	ND	ND	
Final validation	Terbol, 2017–2018																					
	A	10	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	T	6	.022	<.001	<.001	<.001	.153	.153	.153	.153	.153	.153	.153	<.001	.475	<.001	<.001	<.001	<.001	<.001	<.001	<.001
A × T	60	.180	.637	.017	.497	.173	.173	.173	.173	.665	.665	.665	.665	1.000	.950	.950	.950	.950	.950	.950	.950	.146

<sup>a</sup>DFLR, days to flowering; DMAT, days to maturity; PLHT, plant height; RI<sub>HT</sub>, reduction index of plant height; PNPLT, number of pods per plant; SNPLT, number of seeds per plant; GYPLT, grain yield per plant; RI<sub>GY</sub>, reduction index of grain yield per plant; HSW, 100-seed weight; BRPLT, number of branches per plant.

<sup>b</sup>ND, no data.

**T A B L E 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 <sup>a</sup>	Trait <sup>b</sup>									
			DFLR	DMAT	PLHT	RI <sub>HFR</sub>	GYPLT	RI <sub>GY</sub>	PNPLT	SNPLT	BRPLT	HSW
Preliminary screening	Marchouch, 2014–2015	T1	ND <sup>c</sup>	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
		T1	105.4	169.2	51.8	23.4	12.6	29.6	12.9	26.2	ND	ND
	Terbol, 2015–2016	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
		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
Validation trial	Marchouch, 2016–2017	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
		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
	Terbol, 2016–2017	SE	0.8	0.4	0.9	9.8	6.1	5.2	0.9	3.9	ND	ND
		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
Final validation	Terbol, 2017–2018	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
		T1	99.1	169.1	51.1	6.8	19.4	13.4	11.3	23.7	4.4	85.8

<sup>a</sup>T1, Metribuzin 250 g a.i. ha<sup>-1</sup>; T2, Imazethapyr 75 g a.i. ha<sup>-1</sup>; T3, Metribuzin 375 g a.i. ha<sup>-1</sup>; T4, Metribuzin 500 g a.i. ha<sup>-1</sup>; T5, Imazethapyr 112.5 g a.i. ha<sup>-1</sup>; T6, Imazethapyr 150 g a.i. ha<sup>-1</sup>; C, Control.  
<sup>b</sup>DFLR, days to flowering; DMAT, days to maturity; PLHT, plant height; RI<sub>HFR</sub>, reduction index of plant height; GYPLT, grain yield per plant; RI<sub>GY</sub>, 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.  
<sup>c</sup>ND no data.

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).

### 3.5 | 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 39.219% 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 ( $p < .01$ ).

### 3.6 | 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).

## 4 | 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

**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<sub>HT</sub>) and grain yield (RI<sub>GY</sub>) for different levels of herbicide damage in each treatment

Treatment	HDS2 <sup>a</sup>	Preliminary screenings				Validation trials			
		Terbol 2015–2016		Marchouch 2014–2015		Marchouch 2016–2017		Terbol 2016–2017	
		RI <sub>HT</sub> <sup>b</sup>	RI <sub>GY</sub> <sup>c</sup>	RI <sub>HT</sub>	RI <sub>GY</sub>	RI <sub>HT</sub>	RI <sub>GY</sub>	RI <sub>HT</sub>	RI <sub>GY</sub>
Metribuzin, 250 g a.i. ha <sup>-1</sup>	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
	<b>Regression (<i>p</i> value)</b>	<b>&lt;.001</b>	<b>.03</b>	<b>.00</b>	<b>.01</b>	<b>.03</b>	<b>.04</b>	<b>&lt;.001</b>	<b>&lt;.001</b>
	<b>Estimate parameter (a 10<sup>-3</sup>)</b>	<b>52.73***</b>	<b>6.38<sup>a</sup></b>	<b>37.50***</b>	<b>0.91<sup>a</sup></b>	<b>37.90<sup>a</sup></b>	<b>20.40<sup>a</sup></b>	<b>79.80***</b>	<b>24.90***</b>
Imazethapyr, 75 g a.i. ha <sup>-1</sup>	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	-	-	-	-
	<b>Regression (<i>p</i> value)</b>	<b>.76</b>	<b>.59</b>	<b>.87</b>	<b>.24</b>	<b>.46</b>	<b>.01</b>	<b>.01</b>	<b>.06</b>
	<b>Estimate parameter (a 10<sup>-3</sup>)</b>	<b>-2.49</b>	<b>1.81</b>	<b>-0.50</b>	<b>0.24</b>	<b>11.30</b>	<b>42.00<sup>a</sup></b>	<b>37.30*</b>	<b>5.69<sup>a</sup></b>

<sup>a</sup>HDS2, second herbicide damage score. <sup>b</sup>RI<sub>HT</sub>, reduction index of plant height. <sup>c</sup>RI<sub>GY</sub>, 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.



**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 <sub>GYIma100</sub>	RI <sub>GYMet100</sub>	RI <sub>PLHTIma100</sub>	RI <sub>PLHTMet100</sub>
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.

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).

#### 4.1 | 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 imidazole 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.

#### 4.2 | 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)

**TABLE 7** Herbicide damage scores (HDS1 & HDS2), plant height, grain yield per plant (GYPLT), and reduction index (RI) of grain yield (RI<sub>GY</sub>) and plant height (RI<sub>HT</sub>) of the selected faba bean accessions with different origin at Terbol 2017–2018 under different herbicide treatments

Origin	Treatment <sup>a</sup>	Metribuzin			Imazethapyr			RI <sub>HT</sub>	RI <sub>GY</sub>	GYPLT	HDS2	HDS1	HDS2	GYPLT	RI <sub>HT</sub>	RI <sub>GY</sub>	RI <sub>HT</sub>
		HDS1	HDS2	GYPLT	RI <sub>GY</sub>	RI <sub>HT</sub>	HDS1										
VF335 (Russia)	1X	2	1	16.8	3.1	13.3	2	1	25.5	13.3	2	1	25.5	13.3	-51.1	-4.8	
	1.5X	2	2	18.3	-14.4	7.7	2	2	27.1	7.7	2	2	27.1	7.7	-59.7	-11.8	
	2X	2	2	18.1	8.1	12.6	2	1	24.2	12.6	2	1	24.2	12.6	-27.2	-2.3	
FB2568 (France)	1X	2	1	14.2	-36.0	-2.9	1	1	19.1	-2.9	1	1	19.1	-2.9	-75.2	-14.3	
	1.5X	2	1	14.1	-38.2	0.2	2	1	19.7	0.2	2	1	19.7	0.2	-72.9	-7.9	
	2X	2	1	13.9	-34.7	0.4	2	1	17.1	0.4	2	1	17.1	0.4	-23.9	-8.6	
ILB132194 (China)	1X	3	2	24.9	-66.5	5.9	2	1	28.00	5.9	2	1	28.00	5.9	-86.6	-8.2	
	1.5X	2	3	9.4	60.9	8.3	2	1	41.3	8.3	2	1	41.3	8.3	-162.7	-11.0	
	2X	3	3	11.2	28.2	21.6	2	1	26.2	21.6	2	1	26.2	21.6	-66.7	2.9	
FB2574 (France)	1X	2	1	19.7	4.7	8.0	1	1	22.1	8.0	1	1	22.1	8.0	-23.2	-4.1	
	1.5X	2	2	25.8	-40.8	12.6	2	2	24.7	12.6	2	2	24.7	12.6	-35.7	-4.6	
	2X	2	2	22.9	-25.3	14.7	2	1	17.4	14.7	2	1	17.4	14.7	2.7	7.3	
FB1482 (Netherlands)	1X	2	2	13.3	-57.6	-19.1	2	1	22.7	-19.1	2	1	22.7	-19.1	-177.2	-37.4	
	1.5X	3	3	5.2	-1.3	7.2	3	3	18.1	7.2	3	3	18.1	7.2	-112.0	-30.5	
	2X	2	2	4.2	49.5	-2.5	2	2	16.4	-2.5	2	2	16.4	-2.5	-70.9	-29.5	
IG12659 (Ethiopia) <sup>b</sup>	1X	2	2	20.5	36.7	7.8	2	1	29.1	7.8	2	1	29.1	7.8	10.0	-3.9	
	1.5X	2	2	16.4	49.3	7.2	2	1	30.3	7.2	2	1	30.3	7.2	6.5	2.0	
	2X	2	3	22.7	30.0	11.8	2	2	22.9	11.8	2	2	22.9	11.8	29.0	6.5	
ILB1814 (Syria) <sup>b</sup>	1X	2	2	34.4	-4.1	14.9	1	1	37.5	14.9	1	1	37.5	14.9	-13.3	-4.0	
	1.5X	2	2	34.2	-3.4	21.4	1	1	43.8	21.4	1	1	43.8	21.4	-32.5	10.0	
	2X	2	2	35.0	-6.0	25.9	2	2	36.1	25.9	2	2	36.1	25.9	-9.1	13.0	
Flip 86-98FB (Lebanon) <sup>b</sup>	1X	2	2	24.6	30.4	10.0	1	1	30.8	10.0	1	1	30.8	10.0	12.8	-11.1	
	1.5X	2	2	19.8	43.9	4.9	1	1	27.1	4.9	1	1	27.1	4.9	23.5	-16.0	
	2X	2	2	25.1	29.1	9.8	2	2	29.2	9.8	2	2	29.2	9.8	17.5	-2.5	
SE (accession [A])	2.6	25.5	4.4														
SE (treatment [T])	1.8	13.9	2.6														
<i>p</i> value (A)	<.001	<.001	<.001														
<i>p</i> value (T)	<.001	.005	<.001														
<i>p</i> value (A × T)	.235	.658	.324														

<sup>a</sup> 1X, 100% of the recommended dose; 1.5X, 150% of the recommended dose; 2X, 200% of the recommended dose.

<sup>b</sup> Accessions selected at both Terbol and Marchouch locations in 2016–2017 season.

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).

#### 4.3 | 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.

#### 4.4 | 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.

#### 4.5 | 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.

### 5 | 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.

## AUTHOR CONTRIBUTIONS

Lynn Abou Khater conducted experiments at Terbol station in 2015–2016, 2016–2017 and 2017–2018 and wrote the first draft. Diana Nacouzi conducted the field experiment at Terbol during 2015–2016 as part of her master's thesis at Lebanese University jointly with Lynn Abou Khater. Rind Balech contributed to the implementation of the trials and to data management. Somanagouda B. Patil conducted experiments at Marchouch station and reviewed the paper. Diego Rubiales revised the paper. He is Lynn's PhD director. Shiv Kumar contributed in fund raising and reviewed the paper. Fouad Maalouf supervised the implementation of all experiments and made a major contribution to the paper writing and editing. He is Lynn's PhD second director. All authors provided critical feedback to the paper.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## ORCID

Lynn Abou-Khater  <https://orcid.org/0000-0003-4230-8743>

Fouad Maalouf  <https://orcid.org/0000-0002-7642-7102>

Diego Rubiales  <https://orcid.org/0000-0001-9644-8616>

Shiv Kumar  <https://orcid.org/0000-0001-8407-3562>

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