UNIVERSIDAD DE CÓRDOBA

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

TESIS DOCTORAL

Mapeo por Asociacion de tolerancia a herbicidas en lenteja para tolerancia a herbicidas.

Genome wide association study (GWAS) for herbicide tolerance in lentils (*Lens culinaris*).

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Noviembre de 2023

TITULO: *Genome wide association study (GWAS) for herbicide tolerance in lentils (Lens culinaris)*

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UNIVERSIDAD DE CÓRDOBA

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

PhD Thesis

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Genome wide association study (GWAS) for herbicide tolerance in lentils (Lens culinaris L.).

INFORME RAZONADO DE LAS/LOS DIRECTORAS/ES DE LA TESIS (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 este tesis docotoral se han desarrollado entre 2014 y 2020 en los estaciones de experimentales de ICARDA en Marruecos y Libano. La doctoranda ha realizado estancias breves en Córdoba, cumpliendo con los requisitos de doctorado y tutela académica. Entre 2021 y 2022 se han analizado los datos y redactado los capítulos resultantes siempre en comunicación directa y fluida con sus codirectores, uno de los cuales estaba en Córdoba y el otro en el Libano. Como resultado los cuatro capítulos han sido publicados o están en prensa siendo en todos ellos la doctoranda primera autora:

Capítulo 1: Balech et al., (2024). Weeds Management in Cool Season Legumes. Submitted.

Capítulo 2: Balech et al., (2022). Evaluation of performance and stability of new sources for tolerance to post-emergence herbicides in lentil (Lens culinaris ssp. culinaris Medik.). Crop and Pasture Science, 73(11): 1264-1278. https://doi.org/10.1071/CP21810, IF2023= 1.9, Ranking 25/58 in Agriculture, Multidisciplinary (Q2).

Capítulo 3: Balech et al. (2023). Assessing the Stability of Herbicide-Tolerant Lentil Accessions (Lens culinaris Medik.) under Diverse Environments. Plants, 12(4): 854. https://doi.org/10.3390/plants12040854, IF2022=4.5, Ranking 39/238, Plant Science (Q1).

Capítulo 4: Balech et al., (2023). Identification of novel genes associated with herbicide tolerance in Lentil (Lens culinaris ssp. culinaris Medik.). Scientific Reports, en prensa, IF2022=4.6, Ranking 17/73, Multidisciplinary Sciences (Q1).

Por todo ello, se autoriza la presentación de la tesis doctoral.

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Genome wide association study (GWAS) for herbicide tolerance in lentils (Lens culinaris).

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Por la presente ratifico el informe favorable emitido por los directores de la tesis doctoral. La parte experimental de la tesis ha sido realizada en el ICARDA, centro internacional de reconocido prestigio y co-supervisada por los doctores Fouad Maalouf , Shiv Kumar (ICARDA) y Diego Rubiales (IAS-CSIC), habiendo dado lugar a cuatro artículos.

Por todo ello, se autoriza la presentación de la tesis doctoral.

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ACKNOWLEDGMENTS

When I embarked on my PhD journey five years ago, I knew little about the profound experiences and challenges that lay ahead. After several years in professional practice at the International Center for Agricultural Research in the Dry Areas (ICARDA) and then joining back the university environment in parallel with my job at ICARDA, was both a daunting and exhilarating experience. Therefore, I would like to express my sincere gratitude to the people who played a significant role and supported me throughout all this journey as none of this would have been possible without their presence.

First and foremost, I would like to thank my PhD supervisors, Prof. Diego Rubiales, Dr. Fouad Maalouf and Dr. Shiv Kumar. Their constant support, guidance, and encouragement was invaluable throughout the entire journey. I am profoundly grateful for the immeasurable contributions they made to my development. Prof. Diego Rubiales played a pivotal role in helping me establish myself within the academic community and he always shared generously his extensive academic and professional knowledge. Dr. Shiv Agrawal and Dr. Fouad Maalouf, to whom I am truly thankful for giving me the opportunity to pursue my PhD while being part of ICARDA’s team at the Food Legumes Breeding Program. From the initial stages of refining my research proposal to the final submission of my thesis, I highly valued the follow-up and the encouragement they provided me.

At the end of this work, I would like to thank Dr. Hassan Machlab, the director of ICARDA -Terbol station, for this enriching opportunity and all the facilities they offered me. A special appreciation goes to my colleagues at ICARDA; Dr. Lynn Abou-Khater, Miss Marie Wehbe and Mr. Ghazi Khatib; for their continuous support, and valuable help throughout this long journey.

I would also like to express my gratitude to my PhD thesis committee members for their time and effort deployed to reading, and evaluating my thesis, and for providing me valuable feedback. Your insights allow me to enrich my work.

Lastly, I want to express my heartfelt and deepest gratitude to my family and friends. Thank you for always being there, accompanying me through the highs and downs of this academic journey. Your support and belief in me have been a source of strength and motivation. I hope you are aware of and proud of the significant role you have played in shaping my path.

ABSTRACT

Cool Season grain legumes are vulnerable to the presence of weeds, resulting in significant yield and economic losses all over the world annually. Among these legumes, lentil crop (*Lens culinaris* ssp.) is one of the most susceptible crops to this biotic stress leading potentially to 100% losses in some cases. To lessen and to cease the impact of this problem, herbicide application is considered as the most effective tool. However, optimal control of weeds requires a post-emergence application of herbicides which leads to severe phytotoxicity symptoms in lentil crop. Hence, post-emergence tolerance in lentil crops has become mandatory. Additionally, the overreliance on the application of the same herbicide in a cropping system led to the occurrence of weed resistance against herbicides. Thus, for an optimal chemical control of weeds, the selection for tolerance to at least two herbicides applied alternatively in post-emergence stage has become crucial.

Therefore, a set of 221 lentil accessions were screened at the experimental research station of the International Center for Agricultural Research in the Dry Areas, Terbol (Lebanon) over four crop seasons (2014/15–2018/19). The preliminary screening of 221 lentil accessions during 2014/15 season, at 150% of the recommended dose of imazethapyr (112.5 g active ingredient/ha (g a.i./ha)) or metribuzin (315 g a.i./ha /ha) resulted in the selection of 38 accessions. These selected lines were screened at 100% and 150% of the recommended doses of imazethapyr or metribuzin. Based on the phenological, and yield components data and the stability analysis, four accessions were found independently tolerant to metribuzin and imazethapyr; two of them (IG4400 and IG323) adapted to high rainfall environments and two others (IG5722 and IG4605) adapted to low rainfall environments.

Additionally, to enhance the selection process of a breeding program, the adaptability and stability of 42 lentil accessions with different degrees of tolerance to imazethapyr and metribuzin were investigated using five stability parameters under eight different environments from 2015 to 2019, under three herbicide treatments imazethapyr: 75 (g a.i. ha^{-1}); metribuzin: 210 (g a.i. ha-1) and without herbicide treatment at two locations; Marchouch, Morocco and Terbol, Lebanon. In this study, the adopted stability parameters are Cultivar Superiority, Finlay–Wilkinson, Shukla, Static Stability, and Wricke's Ecovalence and each environment is the result of the interaction of seasons, locations, and herbicide treatments. Results showed that Genotype–Environment (GE) interaction was found significant for days to flowering (DF), days to maturity (DM), and seed yield per plant (SY). The tested accessions were ranked

differently confirming that the adaptability and stability of a group of lentil accessions should be studied using a combination of stability parameters. GGE biplot of the SY trait showed that cool and high rainfall environments are ideal for testing the agronomic performance of tolerant accessions and IG70056(38) was identified as a superior line having a high and stable yield across environments.

Finally, to identify marker traits associated with herbicide tolerance, the meta-GWAS analysis was deployed using genome-wide single nucleotide polymorphism (SNP). A set of 292 lentil accessions were genotyped and phenotyped under different dosages of metribuzin and imazethapyr, during two seasons at Marchouch, Morocco and Terbol, Lebanon. A total of 10271 SNPs markers uniformly distributed along the lentil genome were assayed using Multispecies Pulse SNP chip developed at Agriculture Victoria, Melbourne. 36 SNPs were detected highly associated with phenological and yield components traits under herbicide treatments and eighteen of them were located within the genes. Moreover, gene annotation showed that four SNPs of Peptide/nitrate transporter type I/II extracellular region ABC transporter related, Allantoinase / Dihydroorotase, Biotin carboxyl carrier acetyl-CoA carboxylase, and Myelodysplasia-myeloid leukemia factor 1-interacting protein were found to be highly significantly associated with herbicide tolerance traits. The identified SNPs could be used to promote marker assisted selection programs which will enhance and facilitate selections for herbicide tolerance in lentil crop.

RESUMEN

Las leguminosas son muy vulnerables a la infestación de malas hierbas, lo que resulta en importantes pérdidas económicas y de rendimiento en todo el mundo anualmente. Entre ellas, destaca la lenteja (*Lens culinaris*) por las graves pérdidas que puede sufrir. El control con herbicidas de post-emergencia es complicado, debido a la baja tolerancia a los herbicidas disponibles que causan problemas de fitotoxicidad. Además, el desarrollo de resistencia a estos herbicidas en las malas hierbas hace necesario la rotación de herbicidas. Por lo tanto, para un control químico óptimo de malas hierbas en lenteja es recomendable la selección de tolerancia a al menos dos herbicidas aplicados alternativamente en la etapa de postemergencia. Para resolver esta problemática se plantearon tres objetivos principales en este estudio:

1) identificar variabilidad para tolerancias a dos herbicidas de amplio espectro (imazetapir y metribuzina) aplicados en postemergencia.

2) evaluar el rendimiento y la estabilidad del rendimiento de las líneas seleccionadas tolerantes a herbicidas en diferentes ambientes, para identificar el ambiente ideal para la selección y el genotipo ganador ideal que tenga un alto rendimiento medio y una alta estabilidad en todos los ambientes.

3) identificar marcadores moleculares (SNP) asociados significativamente con la tolerancia a imazetapir y metribuzina mediante meta-GWAS. Esto nos ayudará a localizar el gen de la tolerancia y descifrar las asociaciones y mecanismos de tolerancia a herbicidas entre los marcadores SNPs detectados en las regiones genómicas y los rasgos fenotípicos.

Las evaluaciones se realizaron en campo sembrando un conjunto de 221 genotipos de lenteja en ICARDA (Centro Internacional de Investigación Agrícola en las Zonas Áridas), Terbol (Líbano), durante cuatro campañas agrícolas consecutivas (2014/15–2018/19). En la primera campaña (2014/15) se hizo una selección preliminar usando el 150% de la dosis recomendada de imazetapir (112,5 g de ingrediente activo/ha (g ia/ha) o de metribuzina (315 g ia/ha/ha), lo que permitió identificar 38 genotipos prometedores. Estos fueron después estudiados usando el 100% y 150% de las dosis recomendadas de imazetapir o metribuzina. Con base en los datos de los componentes fenológicos y de rendimiento y el análisis de estabilidad, se encontraron cuatro genotipos tolerantes, a ambos herbicidas, dos de ellos (IG4400 e IG323) adaptados a ambientes de alta precipitación y otros dos (IG5722 e IG4605) adaptados a ambientes de baja precipitación.

Además, para mejorar el proceso de selección de un programa de mejora, se investigó la adaptabilidad y estabilidad de 42 accesiones de lentejas con diferentes grados de tolerancia a imazetapir y metribuzina utilizando cinco parámetros de estabilidad en ocho ambientes diferentes de 2015 a 2019, bajo tres tratamientos con herbicidas imazetapir: 75 (g ia/ha); metribuzina: 210 (g ia/ha) y sin tratamiento herbicida en dos localidades; Marchouch, Marruecos y Terbol, Líbano. En este estudio, los parámetros de estabilidad adoptados fueron la Superioridad del Cultivar, Finlay-Wilkinson, Shukla, Estabilidad Estática y Ecovalencia de Wricke, siendo cada ambiente el resultado de la interacción de estaciones, ubicaciones y tratamientos herbicidas. Los resultados mostraron que la interacción Genotipo-Ambiente (GE) fue significativa para los días hasta la floración (DF), los días hasta la madurez (DM) y el rendimiento de semillas por planta (SY). Las muestras probadas se clasificaron de manera diferente, lo que confirma que la adaptabilidad y estabilidad de un grupo de muestras de lentejas deben estudiarse utilizando una combinación de parámetros de estabilidad. El biplot GGE del rasgo SY mostró que los ambientes frescos y con abundantes precipitaciones son ideales para probar el desempeño agronómico de accesiones tolerantes y se identificó IG70056(38) como una línea superior que tiene un rendimiento alto y estable en todos los ambientes.

Finalmente, se identificaron los rasgos marcadores asociados con la tolerancia a herbicidas mediante análisis meta-GWAS. Para ello se genotiparon y fenotiparon un conjunto de 292 genotipos de lenteja bajo diferentes dosis de metribuzina e imazetapir, durante dos temporadas en Marchouch (Marruecos) y Terbol (Líbano). Se analizaron un total de 10271 marcadores SNP distribuidos uniformemente a lo largo del genoma de la lenteja utilizando el chip Multispecies Pulse SNP desarrollado en Agriculture Victoria, Melbourne. Se detectaron 36 SNPs altamente asociados con rasgos de componentes fenológicos y de rendimiento bajo tratamientos con herbicidas y dieciocho de ellos estaban ubicados dentro de los genes. Además, la anotación genética mostró que cuatro SNPs de la región extracelular tipo I/II del transportador de péptido/nitrato relacionado con el transportador ABC, la alantoinasa/dihidroorotasa, la acetil-CoA carboxilasa portadora de biotina carboxilo y la proteína que interactúa con el factor 1 de mielodisplasia-leucemia mieloide eran altamente significativamente asociado con rasgos de tolerancia a herbicidas. Los SNPs identificados podrían usarse para promover programas de selección asistida por marcadores que mejorarán y facilitarán las selecciones para la tolerancia a herbicidas en el cultivo de lentejas.

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CHAPTER I
General Introduction
Weeds Management in Cool Season Grain

1. Cool season grain legumes overview and major constraint

Cool season grain legumes (CSGL) belong to the family of Fabaceae (or Leguminosae). They have a long and rich history dating back to the neolithic era and are believed to be among the earliest crops cultivated 9000 years BC (Aykroyd and Doughty 1982) for human and animal consumption. Based on their adaptation into various geographical and climatic regions, CSGL are primarily cultivated as winter crops in Mediterranean environments and on high elevations in subtropical regions, and as spring crops in cooler regions. The major CSGL crops are dry pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris*), lupins (*Lupinus* spp.), grass pea (*Lathyrus sativus* L.) and common vetch (*Vicia sativa* L.) (Andrews and Hodge 2010). These crops are increasingly gaining recognition for their importance in enhancing human health, bolstering livestock production, improving soil fertility, and reducing greenhouse gas emissions. Additionally, they hold significance as a valuable source of nutrition, contribute to ensuring food security and have a positive impact on local economies. Chickpea and lentil are used primarily for food whereas pea, faba bean, and grass pea have double use, both for human food and animal feed, depending on the region and cultivars. In fact, grains of these crops contain 25-39% protein content (Vollmann 2016) in comparison to 10-15% in cereals (Singh and Singh 1992; Akibode and Maredia 2011) are good sources of complex carbohydrates, dietary fiber, vitamins, and minerals (McPhee and Muehlbauer 2002; Gebrelibanos *et al.* 2013) Consumption of legumes also plays a major role in preventing and treating several diseases due to low glycemic index (Boye *et al.* 2010; Kalogeropoulos *et al.* 2010; Carvalho *et al.* 2011). These crops are also used for animal nutrition as feed and fodder in various farming systems due to their abundant polysaccharides, amino acids, and carbohydrate contents (Jezierny *et al.* 2010; White *et al.* 2015; Gogoi *et al.* 2018) CSGL crops play a crucial role in enhancing soil health due to their ability to fix atmospheric nitrogen and enhancing soil carbon sequestration (Kirkegaard *et al.* 2008; Peoples *et al.* 2009; Hossain *et al.* 2016).

It is forecasted that by 2050, the world population will increase by 2.3 billion people which requires 2.4% increase in the yield of the major legume and cereal crops (Nawaz and Chung 2020). Moreover, the protein-calorie malnutrition (PCM) is a predominant nutritional challenge in many developing countries across the globe (Iqbal *et al.* 2006; Gebrelibanos *et al.* 2013) exploring an alternative protein source has generated a growing fascination in incorporating grain legumes into diets, given their valuable contribution as a vital plant-based protein source. The harvested area under CSGL crops has increased substantially from 28.1 Mha (million hectares) in 1990 to 34.5 Mha in 2021. This expansion was accompanied by a significant increase in global production, from 32.9 MT (million tons) to 41.8 MT (Figure 1) (FAOSTAT 2023). This increase in production might be attributed to recent advancements made in improved varieties and production technologies dedicated to CSGL crops. Nevertheless, the growth during the last three decades was not at the same pace due to a wide range of biotic and abiotic stresses which prevent the farmers from exploiting and benefiting from their potential. Among biotic stresses, weeds are the most damaging causing significant yield and economic losses to CSGL production globally. In this paper, we reviewed major weeds and management techniques, highlighting the importance of establishing an integrated weed management strategy by incorporating herbicide tolerant varieties.

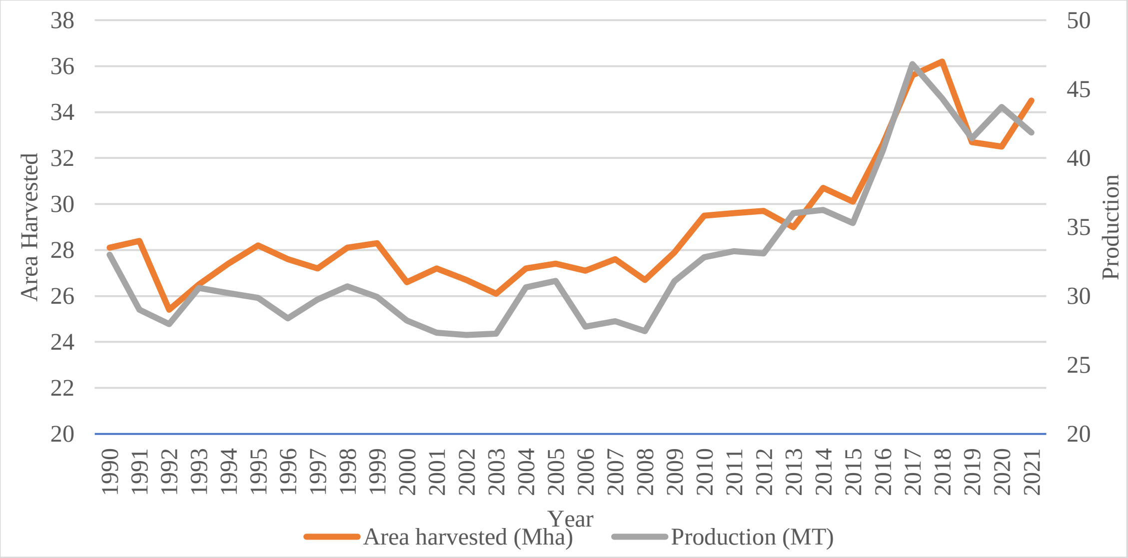

Figure 1. Global trends in area and production of major cool season grain legumes between 1990 and 2021; Mha: million hectares, MT: million tons.

2. Major weeds in legume field

CSGL crops are invaded by a wide range of weeds due to their phytomorphology and limited adoption of improved technologies (Siddique *et al.* 2012). Intensity of weed infestation is, however, influenced by the climate, soil type, crop rotation, weed seed bank in the field, and sowing time (Knott and Halila 1988). In early development stages, CSGL crops grow slowly and thus are poor weed competitors which cause yield losses varying between 20 and 80%. The intensity of losses depends on several factors including crop variety, growth stage, ground cover, sowing time, plant density, inter-row spacing, and the level and type of weed infestation, management practices, soil fertility and moisture level (Knott and Halila 1988; Mohamed *et al.* 1997; Siddique *et al.* 2012). For instance, most pea varieties lodge during vegetative growth stages, causing weeds to grow through the crop and covering it, which eventually causes dramatical yield losses. Faba bean is majorly cultivated in the Mediterranean areas known with high rainfall precipitation during winter which induces severe infestation of various weeds affecting its growth and yield. Chickpea and lentil are also poor weed competitors due to their slow emergence, short plant height and late canopy cover (Yenish 2007). Weeds compete with lentil due to its shallow roots, poor early vigor, and open growth habit which easily stimulates the emergence and development of a surfeit of weeds (Smitchger *et al.* 2012). The estimated yield losses caused by weeds in lentils may reach 100% in highly infested fields (Erman *et al.* 2004; Tepe *et al.* 2005; Yenish *et al.* 2009).

Weeds interfere with the crop throughout their development cycle competing with them for sunlight, water, nutrients, space and host diseases and pests which affect the production (Rubiales and Fernández Aparicio 2012; Sharma *et al.* 2017a). Furthermore, weeds might release metabolites causing allelopathic effect on the seed germination (Tanveer *et al.* 2010; Soliman *et al.* 2017). Also, weeds that germinate and grow at flowering and maturity stages are of high importance during the harvesting process (Brand *et al.* 2007) especially if the seeds of legumes are contaminated with weeds' residuals having similar seed size, gravity and color (Knott and Halila 1988). For instance, wild *Vicia* spp. can be a major problem for the harvest of lentil crop causing sometimes to reject an entire crop. In some cases, the use of desiccants at the pre-harvesting stage is an option to reduce this weedy contamination, however, increase in the cost of production prevents the small holder farmers from opting for this option.

2.1.Annual and perennial weeds

The major weeds found in legume fields can be annual, perennial, and parasitic. Annual and perennial weeds are generally non-specific to a CSGL crop, unlike parasitic weeds, which target a particular host crop. Annual and perennial weeds invade CSGL crops and cause significant (50-70%) yield reductions (Ekeleme 2009). CSGL crops tend to increase the infestation of some annual and perennial weeds due to their ability of nitrogen fixation (Permin 1982; Melander *et al.* 2016). For instance, the populations of *Elytrigia repens* in northern Europe, has increased four times more in crop mixture of spring barley (*Hordeum vulgare*)-grain legumes than in cereal sole crop (Rasmussen *et al.* 2014). In general, the late germinating weeds during the vegetative and pod filling stages of the crop compete with the crop for dry matter and protein which directly affects the seed size and quality. A highly infested field of legume crop with weeds disturb not only harvesting but also the produce as some wild leguminous weedy species have seeds similar in shape and size to lentil seeds, contaminating the seed lots (Figure 2) which lowers the quality and increases the cost of the produce as the separation of seeds is very difficult (Brand *et al.* 2007). Some weeds like prickly lettuce (*Lactuca serriola*) are present at the harvesting stage, they produce a sticky substance causing some weedy residues to adhere to the seeds of the crop (Yenish 2007).

Figure 2. Similarities in seed size, shape, and color between lentil seeds and *Vicia sativa*.

Annual and perennial weed infestation is subject to crop specificity and environmental conditions. Some annual and perennial weeds are found in several CSGL fields while others infest specific grain legume crops and are found in specific regions. The most common weeds of the major CSGLs along with their regions are presented in Table 1. For instance, in faba bean, the most dominant annual weed species especially in the Mediterranean and East Africa are *Chenopodium album* (lambsquarters), *Convolvulus arvensis* (field bindweed), *Lolium multiflorum* (annual ryegrass), *Fumaria officinalis* (fumitory, henbit), *Papaver rhoeas* (corn poppy), *Polygonum aviculare* (prostrate knotweed), and *Sinapis arvensis* (wild mustard) (Cubero 1974; Garcia De Arevalo *et al.* 1992; Abou-Khater, Maalouf, and Rubiales 2022). Additionally, drastic economic losses are reported in CSGL crops due to annual and perennial weeds if not managed properly. The annual economic losses due to weeds in the world were reported around 40 billion USD (Monaco *et al.* 2002). For instance, in Australia, economic losses in chickpea due to *Brassica tournefortii* infestation were estimated at 10.6 million AUD annually (Llewellyn *et al.* 2016; Mahajan and Chauhan 2023).

Table 1. Weed flora found the most in major cool season grain legumes.

Species	Crop	Reference	Region
Annual Weeds			
Salsola ruthenica	Chickpea	(Tepe *et al.* 2011)	Southwestern Asia
Centaurea depressa	Chickpea	(Tepe *et al.* 2011)	Southwestern Asia
Heliotropium europaeum	Chickpea	(Tepe *et al.* 2011)	Southwestern Asia
Brassica tournefortii	Chickpea	(Mahajan and Chauhan 2023)	Eastern Australia
Rapistrum rugosum	Chickpea	(Mahajan and Chauhan 2023)	Eastern Australia
***Sisymbrium* spp.**	Chickpea	(Tepe *et al.* 2011; Mahajan and Chauhan 2023)	Southwestern Asia and Eastern Australia
Medicago indica	Chickpea	(Singh and Singh 1992; Nath *et al.* 2018)	South Asia
Melilotus alba	Chickpea	(Singh and Singh 1992; Nath *et al.* 2018)	South Asia
Portulaca oleracea	Chickpea	(Singh and Singh 1992; Kristó *et al.* 2019; Kumar *et al.* 2020)	South Asia and Central Europe
***Veronica* spp.**	Faba Bean	(Karkanis *et al.* 2016)	Southern Europe
Beta vulgaris	Faba Bean	(El-Metwally *et al.* 2017)	Northeast Africa

Ammi majus	Faba Bean	(El-Metwally *et al.* 2017)	Northeast Africa
Anthemis arvensis	Faba Bean	(Karkanis *et al.* 2016)	Southern Europe
Medicago hispida	Faba Bean	(El-Metwally *et al.* 2017)	Northeast Africa
Galeopsis tetrahit	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
Lamium amplexicaule	Faba Bean	(Karkanis *et al.* 2016)	Southern Europe
Fumaria officinalis	Faba Bean	(Garcia De Arevalo *et al.* 1992; Karkanis *et al.* 2016)	Mediterranean area, East Africa, and Southern Europe
Alopecurus myosuroides	Faba Bean	(Karkanis *et al.* 2016)	Southern Europe
***Echinochloa* spp.**	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
***Phalaris* spp.**	Faba Bean	(Karkanis *et al.* 2016; El-Metwally *et al.* 2017)	Northeast Africa and Southern Europe
Hordeum vulgare	Lentil	(Elkoca *et al.* 2005a)	West Asia
Anagalis arvensis	Lentil	(Yadav *et al.* 2013)	South Asia
Galium aparine	Lentil	(Alinejad *et al.* 2020)	Southwestern Asia
Phalaris minor	Lentil	(Yadav *et al.* 2013)	South Asia
Sinapis arvensis	Peas	(Bilalis *et al.* 2015)	Southern Europe
Stellaria media	Peas	(Salonen *et al.* 2005; Wozniak 2012)	Northern and Central Europe
Echinochloa crus	Peas	(Wozniak 2012)	Central Europe
Solanum nigrum	Chickpea, Lentil	(Kumar *et al.* 2017; Kristó *et al.* 2019)	Central Europe and South Asia
Bromus tectorum	Chickpea, lentil	(Tepe *et al.* 2011; Geddes and Pittman 2022)	Southwestern Asia, and Western United States
***Setaria* spp.**	Chickpea, Lentil	(Uludag *et al.* 2008; Fedoruk *et al.* 2011; Fessehaie and Mohammed 2016)	North-Eastern Africa, West Canada, and North America
Sinapis arvensis	Faba Bean, Lentil	(Garcia De Arevalo *et al.* 1992; Ahmadi *et al.* 2016)	Mediterranean area, East Africa, and South Asia
Papaver rhoeas	Faba Bean, Peas	(Garcia De Arevalo *et al.* 1992; Bilalis *et al.* 2015; Karkanis *et al.* 2016)	Southern Europe, Mediterranean area, and East Africa

Lolium spp.	Lentil, Chickpea, Faba Bean	(Garcia De Arevalo *et al.* 1992; Fessehaie and Mohammed 2016; Karkanis *et al.* 2016; El-Metwally *et al.* 2017; Brunton *et al.* 2018)	Northeast Africa, Southern Europe, Southern Australia, and Mediterranean area
Avena spp.	Lentil, Chickpea, Faba Bean	(Elkoca *et al.* 2005a; Fedoruk *et al.* 2011; Karkanis *et al.* 2016; Mahajan *et al.* 2022)	Western Canada, West Asia, Australia, and Southern Europe
Amaranthus spp.	Lentil, Chickpea, Peas	(Elkoca *et al.* 2005a; Tepe *et al.* 2011; Wozniak 2012)	West Asia, Central Europe, and Southwestern Asia
Chenopodium album	Lentil, Chickpea, Faba Bean, Peas	(Garcia De Arevalo *et al.* 1992; Elkoca *et al.* 2005a; Salonen *et al.* 2005; Mukherjee 2007; Yadav *et al.* 2013; Karkanis *et al.* 2016; El-Metwally *et al.* 2017; Kumar *et al.* 2017; Kristó *et al.* 2019; Alinejad *et al.* 2020; Romaneckas *et al.* 2021)	Asia, Europe, Mediterranean area, East Africa, North America, and Australia
Perennial Weeds			
Taraxacum officinale	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
Plantago major	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
Rumex dentatus	Faba Bean	(El-Metwally *et al.* 2017)	Northeast Africa
Elytrigia repens	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
Persicaria lapathifolia	Faba Bean	(Romaneckas *et al.* 2021)	Northern Europe
Cyperus rotundus	Lentil	(Kumar *et al.* 2017)	South Asia
Cynodon dactylon	Lentil	(Kumar *et al.* 2017)	South Asia
Equisetum arvense	Peas	(Salonen *et al.* 2005)	Northern Europe
Elymus repens	Peas	(Salonen *et al.* 2005; Wozniak 2012)	Northern and Central Europe
Polygonum spp.	Chickpea, Faba Bean, Lentil	(Garcia De Arevalo *et al.* 1992; Elkoca *et al.* 2005a; Khan *et al.* 2011; El-Metwally *et al.* 2017)	Mediterranean area, East Africa, Northeast Africa, and South and West Asia
Sonchus arvensis	Chickpea, Faba Bean, Peas	(Salonen *et al.* 2005; Khan *et al.* 2011; Karkanis *et al.* 2016; Romaneckas *et al.* 2021)	South Asia, South and North Europe

Cirsium arvense	Lentil, Chickpea, Faba Bean, Peas	(Elkoca *et al.* 2005a; Khan *et al.* 2011; Wozniak 2012; Karkanis *et al.* 2016; Romaneckas *et al.* 2021)	South and West Asia, Europe
Convolvulus arvensis	Lentil, Chickpea, Faba Bean, Peas	(Garcia De Arevalo *et al.* 1992; Elkoca *et al.* 2005a; Wozniak 2012; Yadav *et al.* 2013; Amini *et al.* 2015; Kristó *et al.* 2019)	South and West Asia, Central Europe, Mediterranean area, and East Africa
Parasitic Weeds			
Pelipanche aegyptiaca	Lentil, Faba Bean	(Sauerborn 1991; Rubiales, Fernández-Aparicio, *et al.* 2009; Pérez-de-Luque *et al.* 2010)	Middle East and Mediterranean area
Cuscuta campestris	Lentil, Peas, Chickpea	(Mishra *et al.* 2005; Mishra 2009)	South Asia
Orobanche crenata	Lentil, Chickpea, Faba Bean, Peas	(Farah and Al-Abdulsalam 2004; Joel 2009; Restuccia *et al.* 2009)	Middle East, Northern Africa, Southern Europe, and Western Asia
Orobanche foetida	Lentil, Chickpea, Faba Bean, Peas	(Farah and Al-Abdulsalam 2004; Vaz Patto *et al.* 2008; Mishra 2009; Rubiales *et al.* 2014)	Western Mediterranean area, North Africa, Middle East, South Europe, and West Asia

2.2.Parasitic weeds

In general, the most economically damaging weeds for CSGL crops are broomrapes which are parasitic plants completely devoid of chlorophyll that infect the roots of dicotyledonous plants (Rubiales 2023). For instance, these root parasites belonging to the genera *Orobanche* are widely spread especially in Mediterranean regions of Asia, Africa, and southern and eastern Europe (Fernandez-Aparicio *et al.* 2007; Rubiales and Fernández Aparicio 2012). Several broomrape species such as *O. crenata*, *O. foetida*, and *O. aegyptiaca* (syn. *Phelipanche aegyptiaca*) are of holoparasitic weeds that infect various legume crops while pea (*Pisum sativum*) is infected only by *O. crenata* and escapes easily to *O. foetida* and *P. aegyptiaca* (Rubiales and Fernández Aparicio 2012). *O. crenata* attacks both food and forage legumes and widely spread in Middle East, northern Africa, southern Europe, and western Asia countries (Joel 2009; Restuccia *et al.* 2009). Areas under faba bean, peas, and lentils have drastically decreased especially in the Mediterranean region due to widespread infestation of by these weeds (Parker 2009; Maalouf *et al.* 2011; Ozaslan *et al.* 2017). *O. foetida* is widespread in western Mediterranean areas and known to infect wild legumes. Recent studies showed that this specie also infects faba bean crop in Beja region of Tunisia (Vaz Patto *et al.* 2008; Rubiales *et al.* 2014) and in Morocco infecting common vetch (Rubiales, Sadiki, *et al.* 2005). Furthermore, *O. aegyptiaca* is also a harmful pest of legumes and many other vegetable crops in the region of Middle East (Parker 2009). For instance, it has been reported that in the southeast Anatolia region in Turkey, yield losses in lentil can reach 95% depending on the severity of the infestation and the planting date (Sauerborn 1991; Rubiales, Fernández-Aparicio, *et al.* 2009) due to high infestation of fields with *O. aegyptiaca* and *O. crenata* (Aksoy *et al.* 2016). Uludag and Demirci (2005) reported that more than 25% of this region in Turkey is highly infested with these parasitic weeds causing up to 80% average yield loss, leading to an annual economic loss of 60 million Euros. In the Middle East, the annual yield losses are estimated between 1.3 and 2.6 billion dollars due to these parasitic weeds (Aly 2007).

Dodders (*Cuscuta* spp.) are annual stem parasites that belong to the family of Cuscutaceae comprising 175 species distributed all over the world; the most common one is *Cuscuta campestris* (Mishra 2009). Some of them only invade specific crops for which they owe a common name such as flax dodder infests only flax crop. In general, managing weed especially dodders that do not have leaves and grows by curling on the stem or the seeds of the host crop (Lee and Timmons 1958; Mishra 2009). It has been shown that cuscuta is a severe weed that directly extracts nutrients from crops and reduces seed yield and quality. These weeds also have the potential to drop their seeds which have a long shelf life (14 to 60 years) due to their hard seed coat (Mishra 2009). In contrary to broomrapes, seeds of cuscuta require scarification to germinate and not host-root exudates (Benvenuti *et al.* 2005; Yenish 2007). Dodders cause severe yield losses especially in pulses, oilseeds, and fodder crops. For instance, yield reductions reached 87% in lentil, 85.7% in chickpea, 60-70% in alfalfa, and 49.7% in linseed (Moorthy *et al.* 2003; Mishra 2009; Rubiales and Fernández-Aparicio 2012). The intensity of infestation of this parasite depends on its pace to invade the host plant. For instance, Mishra (2009) reported that the increasing densities of cuscuta between 1 and 10 plants/m^2 at Jabalpur, caused significant seed yield reduction in lentil (49.1-84.0%) and chickpea (54.7-98.7%). An experiment led by Farah and Al-Abdulsalam (2004) showed that *C. campestris* decreased the biological yield of lentil, chickpea, faba bean, alfalfa, and pea by more than 50%.

3. Weed control methods

Various mechanical, cultural, biological, and chemical strategies have been adopted to control weeds in CSGL crops, driven by the substantial economic losses and reduced yields they can cause (Parker and Riches 1993). As an illustration, it has been reported by Fernandez et al. (2012) that weed control increased pea yields by an average of 63%, while (Tanveer and Ali 2003) observed that 20 to 50% losses in grain yield can occur if weeds in lentil crop are not adequately managed. The main obstacle in controlling weeds in CSGL crops is the huge stock of weed seeds that remain viable for decades in the soil which will acquire the parasite seeds genetic adaptability and resistance to the newly applied weed management practices. Thus, every time a susceptible host is grown in the infested field, the seeds of the parasites will always be a problem especially if the seedbank is not fully controlled (Rubiales *et al.* 2009). Efforts have been made to improve weed management in CSGLs. When implementing any new strategy into a cropping system to control weeds, it must be socially acceptable, economically feasible, and environmentally safe (Young *et al.* 2000). In addition, the primary focus in managing parasitic weeds has to be on diminishing the soil seedbank, averting seed production and preventing the infection of new regions with parasitic seeds (Rubiales *et al.* 2009).

3.1.Mechanical and manual weeding

Mechanical or manual weeding in CSGL crops is considered as an effective approach to suppress weeds presence and limit their damage. For instance, two hand weeding in lentil resulted in a grain yield higher than when other strategies were applied (Rajput *et al.* 1992) and lentil yields increased by an average of 87% when hand weeded (Fernandez *et al.* 2012). Faba bean grain yield increased 25% when hand weeding was applied 6 weeks after crop emergence (Agegnehu and Fessehaie 2006). In addition, manual weeding increases crop yield by increasing water and nutrient use efficiency (Varma *et al.* 2017). However, manual weeding is becoming increasingly expensive and time consuming all over the world due to shortage of labor and is only practical in low infested soils (Rubiales and Fernández Aparicio 2012; Gogoi *et al.* 2018). Therefore, mechanical weeding has been adopted to replace manual weeding by tilling techniques such as plowing or disking which can bury seeds too deeply and disrupt weed growth at a lower cost. However, excessive tillage can also result in soil erosion and disturb the soil structure (Anderson 2015). Thus, researchers are exploring new cultural tactics to lessen the intensity and impact of tilling (Peigné *et al.* 2007; Mäder and Berner 2012). As a result, techniques such as strip tillage or a crimper-roller that mechanically kills cover crops have been devised, introducing the concept of rotational tillage (Kornecki *et al.* 2009; Brainard *et al.* 2013). Nevertheless, practicing no-till methods was found more advantageous for enhancing soil health (Triplett and Dick 2008) and even a single tillage can severely negate the gained benefits from no-till practices, particularly concerning soil structure (Grandy *et al.*

2006). To sum up, manual and mechanical weeding might seem effective techniques to control weeds but are costly, time consuming and have adverse consequents on soil health, leading to severe soil erosion which indirectly contributes to the reduction of crop yield.

3.2.Soil Solarization

Some weed species have a long shelf life in soil with a low rate of germination, which will enhance the genetic adaptability of weed seeds to any weed management strategy applied. Various approaches such as solarization and fumigation, suicidal germination, catch and trap crops and delayed sowing can be adopted to reduce the seedbank of weeds. For instance, soil solarization technique involves using polyethylene mulch to cover wet soil for a duration of 4 to 8 weeks during the warmest season under sunlight. The temperature in the soil will increase by 9 to 12°C reaching up to 55°C at a depth of 5 cm (Jacobsohn and Kelman 1980). The temperature fluctuations between day and night cause a high rate of lethal effect. Thus, it is proved that solarization is an effective technique to deplete the seedbank of problematic plants (Jacobsohn and Kelman 1980; Sauerborn *et al.* 1989; Abu-Irmaileh 1991; Mauromicale *et al.* 2005). However, this technique is economically not feasible especially for low-value and low-input legume crops and can only be applied in regions having long and sunny summers (Joel *et al.* 2007; Rubiales *et al.* 2009).

3.3.Intercropping

Trap crops or catch crops which are also called false hosts, are plants that stimulate the germination of weed seeds and thus reduce the seed density in the soil. These crops are also known by allelopathic plants which produce biochemicals that influence the germination (Sauerborn 1991; Aksoy *et al.* 2016). Catch crops can be planted in rotation or as intercrop with CSGL. When planting sesame (*Sesamum indicum*) or puddling in rice (*Oryza sativa*), *Cyperus rotundus*, *Phalaris minor* and *Chenopodium album* infestation reduced in the following CSGL crop as has been reported in chickpea (IIPR. 2009; Kumar *et al.* 2016). For an effective intercropping with cool season legumes, it is recommended to include fast growing and short duration crops such as mungbean, urdbean and cowpea which compete aggressively with weeds and reduce its presence by 30-40% (IIPR. 2009; Kumar *et al.* 2016). Additionally, intercropping legume crops with oat (*Avena sativa*), fenugreek (*Trigonella foenum-graecum*) or berseem clover (*Trifolium alexandrinum*) can suppress or deplete the seed germination of orobanche through the released allelochemicals by the roots of the catch crops (Fernandez-Aparicio *et al.* 2007, 2008, 2010, 2013). Aksoy et al. (2016) reported via a trial conducted in

Turkey the effectiveness of using Flax as a trap plant, lentil as a catch plant and some crops of the *Brassicaceae* family as allelopathic plant two months before sowing lentil as a crop to reduce the seed population of *O. crenata*. During two years of experimentation, Flax reduced the number of shoots of *O. crenata* by an average of 62% and its dry weight by 41%; Lentil reduced the number of shoots of *O. crenata* by 50% while the *Brassicaceae* family reduced the number of *O. crenata* shoots by 39%. Additionally, positive results were obtained in controlling weeds when intercropping sorghum with lathyrus at the appropriate ratios (Rad *et al.* 2020). Similarly in central Europe, when compared with lentil monocropping systems, weeds' biomass was also reduced between 24 and 41% depending on the ratios of intercropping used (Wang *et al.* 2012). However, any weed management method is not considered successful if it does not diminish the weed seedbank by at least 95% (Smith and Webb 1996; Gressel 2013; Fernández-Aparicio *et al.* 2020).

3.4.Date of Sowing

Early planting of CSGL crops causes severe infestation by parasitic weeds. Several studies showed that delayed sowing is an effective technique to reduce the infestation of *O. crenata* in chickpea (Rubiales *et al.* 2003), faba bean and common vetch (Pérez-de-Luque *et al.* 2004; Grenz *et al.* 2005) *Striga gesnerioides* in cowpea (Touré *et al.* 1996), and dodder in lentil (Mishra *et al.* 1996). Nevertheless, a delayed sowing reduces yield due to shorter period of vegetative growth and pod filling of the host plant (Yadav 2007). Piggin et al. (2015) showed that in the Middle East region, the combination of no tillage plus early sowing increased grain yield of wheat by 18%, chickpea by 20% and lentil by 15% in comparison with the conventional tillage and late sowing. However, Singh et al. (2014) reported that the delayed sowing of chickpea significantly reduced the infestation and the density of *Melilotus alba, Cynodon dactylon*, *Phalaris minor*, *Chenopodium album,* and *Medicago hispida*. Thus, this technique is not completely consistent and effective and is subject to several factors such as the crop, the weed flora, and the location and its environmental conditions.

3.5.Biological control method

Biological techniques can also be used to control weeds in CSGL crops such as inducing fungal isolates which are pathogenic to parasitic weeds or inducing bacteria that promote plant growth or even through insects. Most of the fungal isolates of *Fusarium* spp. are known for their specificity to the host and longevity in soil. *Ulocladium atrum* and *Ulocladium botrytis* have been found pathogenic to *O. crenata* and the fungus *Myrothecium verrucaria* isolated from faba bean roots have inhibited the germination of *O. crenata* seeds (El-Kassas *et al.* 2005; Müller-Stöver and Kroschel 2005). Additionally, *Trichoderma* and rhizobacteria were found to enhance the growth parameters of faba bean plants without *O. crenata* infection (El-Dabaa and Abd-El-Khair 2020). Sheppard et al. (2006) has also reported that several annual and perennial weeds in Europe can be controlled through classic biological control. Several studies also showed that the specificity of the biological control of these weeds is mandatory such as *Cirsium arvense* which could be controlled through specific rust pathogens (Guske *et al.* 2004; Müller *et al.* 2011). However, biological techniques require the long-term presence of weed host to guarantee the survival of the natural pathogens (Lundkvist and Verwijst 2011).

3.6.Genetic resistance

Several mechanisms of crop resistance to weeds were detected and described in CSGL. Some varieties secrete a limited quantity of exudates that are responsible for inducing the chemotropic effect. Thus, the germination of parasitic weeds would be limited as reported in peas (Pérez-de-Luque *et al.* 2005), chickpea (Rubiales *et al.* 2003, 2004) and vetch (Sillero *et al.* 2005). Another mechanism detected in CSGL crops is pre-haustorial resistance through physical barriers or lignification of endodermal cells (Pérez-de-Luque *et al.* 2005, 2007). A post-haustorial physical resistance has been identified in legumes through blocking the flux of water and nutrients from the host to the parasite (Pérez-de-Luque *et al.* 2005, 2006) and a chemical resistance found in chickpea through secreting toxic exudates to the host leading to the death of the parasitic weed (Rubiales and Fernández Aparicio 2012). As a result, sources of tolerance were found in faba bean to *Orobanche foetida* in Najah (Abbes *et al.* 2007), *O. crenata* in Giza 402 (Nassib *et al.* 1982), and in Giza 843 and Misr 3 (Marwa and Azza 2018). In chickpea, ICCV 95333 and Hazera 4 were found highly resistant to *Cuscuta campestris* (Goldwasser *et al.* 2012). Another type of crop resistance against weeds can be achieved through the competitive ability of crops. In this method, the major indicators to look for are early vigor of the crop, height, biomass, leaf area, tillering and branching capacity, and root growth (Shabbir *et al.* 2021). For instance, faba bean is considered as less competitive with wheat due to its poor vigor at early stages (Lemerle *et al.* 1996; Shabbir *et al.* 2021). Tall genotypes of field pea have a potential of competitiveness against grass weeds more than short genotypes (McDonald 2003). Similar potential of competitiveness was also reported in varieties of faba bean (Shabbir *et al.* 2021) and chickpea (Paolini *et al.* 2006). Moreover, in faba bean and pea, early varieties are a major component in avoiding root parasitic weeds since they reach pod setting stage and maturity before the germination of *O. crenata* infection (Grenz *et al.* 2005; Rubiales *et al.* 2005). Early maturing lentil varieties also helped in applying broad spectrum herbicides to control ryegrass at the maturity stage of lentil, prior to the pod-setting stage of weeds. Early maturity sources have been found in lentil varieties such as ILL590 and ILL4605, ILL7685, ILL6002 (Siddique *et al.* 2013). Though the genetic resistance to weeds found in CSGL crops is incomplete and limited, these findings reduced weeds' infestation and lowered yield and economic losses (Fernández-Aparicio and Rubiales 2012).

3.7.Chemical control

Lately, attention has been drawn to promising herbicides in CSGL crops which is considered as the most effective technique till date. Managing weeds by applying herbicides enhances productivity and facilitates the utilization of resources such as irrigation, fertilizers, as well as plant protection measures by easily applying insecticides and fungicides. In addition, eradicating weeds from growing crops eases the harvesting process and brings out a premium quality of yield exempt of weed seeds. Thus, chemical weed control is promptly adopted under difficult environmental conditions and limited technical sources where manual or mechanical weeding would not be possible. It is a more convenient, less time-consuming, and cost-effective alternative. Nonetheless, it is important to acknowledge several drawbacks of this technique such as environmental pollution, its effects on human and animal health, and phytotoxicity that might occur on the cultivated crops especially if not applied at the right dosage and timing.

In developed countries, herbicides are the most used chemicals with a range of 60 to 70%. Since their introduction in 1940's, herbicides proved to be efficient and capable of controlling weeds almost by 100% (Young *et al.* 2000). The choice of herbicide to be applied is determined by three main factors: (1) if the parasite is attached to the crop, (2) if the applied herbicide is selective or not; herbicides inhibiting photosynthesis cannot control holoparasitic and achlorophyllous parasite as root parasitic weeds start their damage on the crops while still underground so the post-emergence herbicides won't be able to prevent yield losses; (3) if the herbicide applied is able to control the weed without damaging the crop (Wickett *et al.* 2011; Westwood *et al.* 2012; Gressel 2013; Fernández-Aparicio *et al.* 2020).

The major herbicides used in controlling weeds in CSGL crops are glyphosate, imidazolinones, and metribuzin (Joel *et al.* 2007). These herbicides are anti-metabolites that inhibit the work of a specific key enzyme of the targeted weeds and eventually stopping the plant growth and killing it (Singh and Yadav 2012). Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum herbicide that eradicates any perennial, annual and parasitic weed by interrupting the biosynthesis of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which reduce the synthesis of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) (Malik *et al.* 1989; Nandula *et al.* 2005; Rubiales and Fernández Aparicio 2012). These amino acids are primary in the formation of proteins, cell walls, and secondary plant products (Nandula *et al.* 2005; Peel *et al.* 2013). Additionally, the inhibition of EPSPS disturbs the shikimic acid pathway causing the deregulation of plant carbon metabolism (Velini *et al.* 2009). To effectively control orobanche in faba bean, it is recommended to apply glyphosate twice at a low rate (80 g a.i. ha^{-1}) at the flowering stage (Sauerborn *et al.* 1989; Maalouf *et al.* 2021). Imidazolinones family including imazethapyr and several other active ingredients is a class of herbicides that inhibit the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS). ALS enzyme is an essential component for the biosynthesis of branched-chain amino acids in plants. This family of herbicides control a broad spectrum of broadleaf and grass and even some parasitic weeds (Hanson and Thill 2001; Tan *et al.* 2005; Teja *et al.* 2017). For an effective control of some annual and perennial weeds in chickpea it is recommended to apply imazethapyr at 30 g a.i. ha^{-1} 10 days after germination (Rathod *et al.* 2017) and in lentil at 37.5 g a.i. ha^{-1} 25 days after sowing (Teja *et al.* 2017). Triazinone family including Metribuzin ($C_8H_{14}N_4OS$) is a pre- and post-emergence herbicide used to control both broadleaf and grass weeds in several crops (Soltani *et al.* 2005; Simoneaux and Gould 2008). Metribuzin inhibits photosynthesis at photosystem II (PSII) by competing with plastoquinone at the plastoquinone binding site on the D1 protein within the PSII complex (Pan *et al.* 2012). It is highly recommended to apply imazethapyr with trifluralin in fall at 0.4 and 1.4 kg/ha; this combination can control green foxtail (*Setaria viridis*) and wild oat (*Avena fatua*), and wild mustard (*Brassica kaber*) in faba bean (Betts and Morrison 1979). All the previously mentioned herbicides are systemically absorbed and rapidly translocated through foliage and roots of weeds or through plants to the root parasitic weeds (Colquhoun *et al.* 2006; Mao 2015).

The pre-emergence application of these herbicides control weeds at the early stage of crop growth, but weeds germinating after crop emergence menace the crop production (Gaur *et al.* 2013a). Thus, the post-emergence application of these herbicides turned to be mandatory which reduced the infestation of weeds and their seedstock in the soil and is considered as one of the most economical and effective techniques in regions where the epidemiology is high. However, following this application, severe phytotoxicity symptoms on CSGL crops were reported, affecting yield and its components negatively. However, the level of herbicidal phytotoxicity depends on the herbicide and the rate applied, the crop and its variety, the growth stage of the crop and the environmental conditions. For instance, Glyphosate in post-emergence application caused growth retardation, lamination of the apical leaves and chlorosis, and yield reduction varying between 2 and 47% in faba bean (Mesa-Garcia *et al.* 1984; Balech *et al.* 2016). Notably, the yield reduction increased when the applied dose was higher. Furthermore, the crop was more susceptible to glyphosate at the vegetative stage (5 to 7 nodes), and less susceptible at the late-flowering and pod-filling stage (Mesa-Garcia *et al.* 1984; Balech *et al.* 2016). To the best of our knowledge, no publications reported the direct foliar phytotoxicity effect of glyphosate in post-emergence application on lentil and chickpea. Subedi et al. (2017) reported that when glyphosate is applied as a desiccant in lentil at pre-harvest stage, glyphosate affected the seed germination, seedling vigor, milling, and splitting qualities. In chickpea, significant phytotoxicity and cell damage symptoms were observed on roots after glyphosate application (Shahid and Khan 2018).

Several experiments have been conducted to study the phytotoxicity effect of imazethapyr and metribuzin in post-emergence application in CSGL crops. The post-emergence application of imazethapyr or metribuzin on the main crop caused lower vegetative growth with a clear deformation on the newly formed apical leaves, chlorosis, narrowing and burning of leaves (Gaur *et al.* 2013a). Studies in grain legumes have reported phytotoxicity symptoms in lentil (Sharma *et al.* 2018; Mcmurray 2019; McMurray *et al.* 2019; Balech *et al.* 2022, 2023), chickpea (Gaur *et al.* 2013a; Chaturvedi *et al.* 2014; Gupta *et al.* 2018; Izadi-Darbandi *et al.* 2018; Veisi *et al.* 2022), faba bean (Abou-Khater *et al.* 2021a; Abou-Khater *et al.* 2022a), pea (Jurado-Expósito *et al.* 1996; Parihar *et al.* 2017), and soybean (Papiernik *et al.* 2003; Tuti and Das 2010; Sangeetha *et al.* 2012). However, these studies showed a wide range of variability in the phytotoxicity levels on the tested genotypes and selections of new herbicide tolerant accessions were made. This allowed introgression of herbicide tolerance through natural selections or insertion of herbicidal genes of tolerance to widely grown cultivars. Nevertheless, in order to gain broad acceptance, herbicides can't be applied exclusively, and future weed management strategies must be planned to fully align with the specific needs of the environment, society, and economics (Zoschke 1994).

3.7.1. Crop tolerance to herbicides application

To develop herbicide tolerant crops, three main techniques are being adopted; the first is the conventional technique of screening and selecting herbicide tolerant genotypes and cross them with agronomically elite cultivars. The second option is to screen and select cultivars having agronomically desired traits and being tolerant to herbicides at the same time. The third method is to induce mutations biochemically to trigger off tolerance or resistance to herbicides. A crop variety is resistant to an applied herbicide due to three major physiological mechanisms; the variety itself has a limited sensitivity to the molecular site target of the herbicide, its high degradation metabolic ability, and its ability to limit or sequestrate the herbicidal uptake (Duke *et al.* 1991). Sources of natural tolerance to herbicides through conventional techniques were reported in several crops such as in lentil (Sharma *et al.* 2018; Balech *et al.* 2022, 2023; Shivani *et al.* 2022), faba bean (Zeid and Hemeid 2019; Abou-Khater *et al.* 2021a; Abou-Khater *et al.* 2022a), chickpea (Gaur *et al.* 2013a; Prakash *et al.* 2017; Gupta *et al.* 2018), and pea (Al-Khatib *et al.* 1997; Parihar *et al.* 2019). Sources for high tolerance to glyphosate via induced mutations were also reported when screened naturally in a set of lentil germplasm exposed to gamma radiation (300 Gy, 60Co) (Singh *et al.* 2021) and faba bean genotypes exposed to the mutagenic agent, ethyl methane sulphonate (EMS), (Rizwan 2015; Balech *et al.* 2016).

3.7.2. Genomic selection against herbicide treatments

The first step to identify genes imparting tolerance to herbicides in CSGL crops is to screen a set of germplasm for herbicide resistance and locate herbicide-resistant genetic loci through genome-wide associations studies (GWAS) and quantitative trait loci (QTL) mapping. The approach relies on the linkage disequilibrium arising from the association between alleles at a marker site and alleles of an associated genetic factor that regulates the trait, which is deciphered through a significant correlation between the marker and the trait's expression (Sari-Gorla *et al.* 1997). Till date, several highly significant associations between various genes and herbicide tolerant traits have been detected especially for imazethapyr and metribuzin in faba bean (Abou-Khater *et al.* 2022). Similar studies were also reported on metribuzin tolerance in field pea (Javid *et al.* 2017) and on imazethapyr tolerance in chickpea (Sundaram and Singh 2020). The identified QTLs in these studies could be fine mapped to localize the genes accountable for herbicide tolerance which could facilitate their introgression into elite cultivars through a dedicated breeding program. Several genetic studies revealed successful results and helped to improve tolerance to promising herbicides by inserting genes of tolerance into new cultivars.

3.7.3. Genetically modified crops

Resistance to herbicides has been achieved by genetic modification in several crops. Examples of the genetic modification of glyphosate resistance by inducing cp4 epsps (*Agrobacterium tumefaciens* strain CP4) and gat4621 (*Bacillus licheniformis*), goxv247 (*Orchobactrum anthropi* strain LBAA), mepsps (*Zea mays*), or 2mepsps (*Zea mays*) genes in soybean (*Glycine max*), corn (*Zea mays*), wheat (*Triticum aestivum*), cotton (*Gossypium hirsutum*), canola (*Brassica napus*), sugar beet (*Beta vulgaris*), alfalfa (*Medicago sativa*) or potato (*Solanum tuberosum*) (Owen and Zelaya 2005; Duke and Powles 2009) are available. Another target for genetic modification has been zm-hra gene (*Zea mays*) for resistance to Acetolactate synthase- (ALS-) herbicide (imidazolinone herbicides) in corn, wheat, rice, canola, and sunflower (Bedbrook *et al.* 1995; Green *et al.* 2009; Gage *et al.* 2019). Recently, CRISPR-Cas9 base editing and prime editing have also been employed to increase herbicide tolerance in maize by altering Acetolactate Synthase 1 (ZmALS1) and ZmALS2 and in rice, concomitant HDR-mediated repair of the OsALS gene resulted in herbicide resistance (Svitashev *et al.* 2015; Ali *et al.* 2020). Khatib et al. (2007) also reported the efficacy of developing herbicide resistant lentil by the integration of bar gene into agrobacterium strain AgL0 which produced transgenic plants resistant to ammonium glufosinate. However, genetic studies on herbicide tolerance in CSGL crops are limited and further studies about the insertion of these genes of tolerance must be led.

Nevertheless, the usage of herbicide tolerant varieties has turned out to be insufficient to control weeds. When the same herbicide is continually applied, some weeds will become more resilient and dominant in the population which will fortify their resistance toward the applied herbicide (Rizwan 2015). Weeds tolerance to herbicides is developed through two processes: (1) Biotype arising from intensity of selection; a small portion of the same weed population is genetically slightly distinct and can withstand the applied herbicide and (2) Resistance developed through mutations due to frequent exposure of the weed population to the same herbicide (Singh and Yadav 2012). There are two types of resistance to herbicides. Cross resistance is when a weed species is resistant to several herbicides belonging to the same family, and multiple resistance is when weeds develop resistance to several herbicides belonging to different families (Singh and Yadav 2012). Several studies have reported weeds' resistance to herbicides through the previously described processes. For instance, wild oat was found resistant to acetyl CoA carboxylase inhibitors herbicides and *Chenopodium album* and *Amaranthus retroflexus* were found resistant to metamitron herbicide (Adamczewski *et al.* 2019). Moreover, the following weed families invading CSGL crops have the highest number of resistant species to herbicides: Poaceae (80 species), Asteraceae (39), Brassicaceae (22), Amaranthaceae (11), Chenopodiaceae (8), and Polygonaceae (7). Additionally, 66 weeds species were found resistant to imazethapyr and 34 species to glyphosate (Vrbničanin *et al.* 2017). To avoid this problem, screening of varieties resistant to multiple herbicides is crucial; this way, at least two herbicides can be applied alternatively into a cropping system.

4. Integrated weed management

As already demonstrated, several techniques are available to reduce the damages caused by weeds on CSGL crops (Rubiales and Fernández Aparicio 2012) but every technique turns out to be either partially effective or very complicated or too expensive or environmentally unacceptable. Thus, for effective weed control, the use of a single technique alone is not effective. Hence, Integrated Weed Management (IWM) employs a blend of mechanical, cultural, biological, and chemical strategies aiming to control weeds effectively and to improve sustainability of the farming system. Subsequently, the crop productivity will increase, the economic losses will be reduced, risks to human health and damages to the ecosystem will be limited (Brand *et al.* 2007). An integrated weed management takes into consideration the weeds genera, its biology and rate of infestation, the location and the environmental conditions, the financial and technical resources available, the crop type, and its phytomorphology, the application timing, and the available herbicides and regulations. Studies showed that the most time-cost efficient IWM are the ones deploying herbicide tolerant varieties which leads to an effective weed management and limits other environmental concerns (Kishore *et al.* 1992; Knezevic and Cassman 2003; Lamichhane *et al.* 2017). Herbicide tolerant varieties are a new valuable addition to CSGL crops and their adoption is still limited so far. There has been a lot of controversy regarding the integration of this technique in IWM due to health and environmental impacts and weeds' resistance to the applied herbicide. However, if mechanical weed control is suggested as a fast and effective alternative, this method also cause soil erosion and is energy intensive which also contribute to environmental pollution (Kishore *et al.* 1992). Therefore, as already mentioned, a unique weed management technique is not recommended in a farming system. Alternatively, IWM using herbicide tolerant varieties to desirable herbicides is the best option for controlling weeds in CSL crops. So much work has been done in soybeans, corn, cotton, and canola (Norsworthy 2003; Stewart *et al.* 2011; Riar *et al.* 2013; Wallace *et al.* 2018) from which weed management strategies could be tested and applied into CSGL crops. Growers all over the world of these crops have easily integrated herbicide tolerant varieties to their IWM programs due to efficacy in controlling a wide spectrum of weeds germinating after emergence without causing any undesirable injuries to the main crop and without worrying about crop rotation limitations. Moreover, such systems encourage farmers to adopt new beneficial practices such as conservative tillage and narrow row spacing (Carpenter and Gianessi 1999). In North America by 2001, Glyphosate-resistant crops acreage have increased in corn (15%), soybean (80%), cotton (57%) and canola (60%) (Knezevic and Cassman 2003). More than 55% of total acreage of soybean was also replaced by glyphosate-resistant varieties in Argentina (Duke *et al.* 1991). However, as stated before, weeds' resistance to herbicide is a major limitation for integrating this technique in IWM due to the repeated application of the same herbicide having the same mode of action. Therefore, IWM implementation along with the adoption of varieties resistant to multiple herbicides is the optimal strategy for weed control in CSGL crops (Singh and Yadav 2012).

5. Conclusion

It can be concluded that the most effective approach to sustainably manage weeds and enhance food legume production involves integrating mechanical, cultural, biological, and chemical methods of weed management. However, given the prevalent dependence on herbicides in many countries due to their cost-effectiveness and efficient weed control it is imperative for weed management researchers to prioritize the development of herbicide-tolerant varieties. In recent years, continuous use of selective herbicides has led to the development of herbicide resistance in weeds. In CSGL crops, resistance of weeds to applied herbicides is a severe and escalating problem all over the world. We observed through our review that limited efforts are made to developing herbicide tolerant varieties in CSGL crops, especially in lentil varieties combining tolerance to two or more herbicides to alternate between the applications for a better and quicker weed control. Therefore, developing and using multi-herbicides tolerant variety along with other techniques would serve the purpose of controlling weeds effectively. Thus, our efforts are underway to develop lentil varieties tolerant to multiple broad-spectrum herbicides, imazethapyr and metribuzin applied at the post-emergence stage. In addition, we evaluated the performance and stability of the newly selected sources for tolerance to imazethapyr and metribuzin at different locations. Finally, the screened germplasm was genotyped through the genome wide association mapping technique (GWAS) and new genes of tolerance to both herbicides were identified in lentils.

6. References

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Thesis Objectives

Chapter II: Identify promising lentil varieties tolerant to two broad spectrum herbicides; imazethapyr and metribuzin applied at the post-emergence stage, to evaluate and identify the best parameter that should be adopted for selection and to evaluate the performance and stability of the selected varieties under diverse environments.

Chapter III: Evaluate the performance and yield stability of the new selected sources of tolerance to imazethapyr and metribuzin under multi-environments, to identify the ideal environment for selection and the ideal winning genotype having high mean yield and high stability across environments.

Chapter IV: The purpose of this study is to deploy meta-GWAS analysis to identify SNPs markers significantly associated with tolerance to herbicides of a set of lentil genotypes that was screened for tolerance to imazethapyr and metribuzin under diverse environments. This will help us locate the gene of tolerance and decipher the associations and mechanisms of tolerance to herbicides between the detected SNPs markers on the genomic regions and the phenotypic traits.

Chapter II

Evaluation of performance and stability of new sources for tolerance to post-emergence herbicides in lentil (*Lens culinaris* ssp. *culinaris* Medik.)

1. Introduction

Lentil (*Lens culinaris* ssp. *culinaris* Medik.) is an important cool-season food legume crop worldwide. It has been incorporated for many decades in the culinary traditions of several countries especially in the Mediterranean, West Asia, Sub-Saharan Africa, and South Asia regions for being highly rich in protein (26%), prebiotics and micronutrients (Kumar *et al.* 2014). It is considered as a key option for sustainable intensification and diversification of cereal-based cropping systems due to its positive effect on cereal crops, adaptation to local conditions, ability to fix atmospheric nitrogen, and capability to reduce carbon footprints and water use (Joshi *et al.* 2017; Ouji and Mouelhi 2017). The latest triennium average suggests that the global production of lentil is 6.28 million tons from 5.40 million ha area with an average productivity of 1163 kg/ha (FAOSTAT 2021). The top ten lentil producing countries, namely Canada, India, Australia, Turkey, United States of America, Nepal, Kazakhstan, Bangladesh, Russian Federation, and China, collectively produced more than 92% of global output (FAOSTAT 2021). With rising interest in plant-based protein among health-conscious people, the gap between demand and supply of protein-rich lentils is increasing (Rubiales, Moral, *et al.* 2021). To bridge this gap, there is a dire need to increase the productivity per unit area by adopting improved varieties and crop management practices.

Lentil is a poor weed competitor due to its shallow roots, poor early vigour, and slow vegetative growth. Its open growth habit easily stimulates the emergence and development of a plethora of weeds at early crop growth stages (Smitchger *et al.* 2012) especially in cool season environments. The major annual broadleaves weeds competing with lentil are *Centaurea balsamita*, *Ranunculus arvensis*, *Cephalaria syriaca, Lactuca serriola, Sonchus oleraceus, Sinapis arvensis* and *Setaria viridis* (Wall and McMullan 1994; Erman *et al.* 2004; Merriam *et al.* 2021). The estimated yield losses caused by these annual weeds vary from 20 to 80% and may reach 100% in highly infested fields (Erman *et al.* 2004; Tepe *et al.* 2005) depending on the environmental conditions, and density and diversity of weed species (Yadav *et al.* 2007). On the other hand, the parasitic weeds affecting lentil production are broomrapes (mainly

Orobanche crenata, and *O. aegyptiaca*) and dodders (mainly *Cuscuta campestris*), which can cause severe yield damages of up to 95%, especially in North Africa and Western and Central Asia (Rubiales and Fernández-Aparicio 2012).

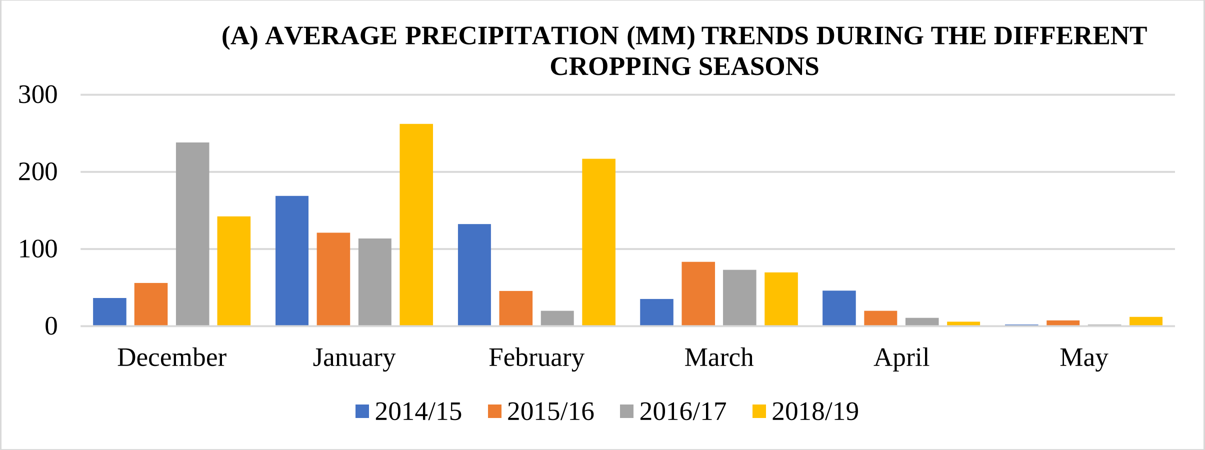
Several weed management practices such as manual weeding, late sowing, higher plant densities, soil sterilization, fertilization and irrigation scheduling are suggested to control weeds in lentil fields (Brand *et al.* 2007). Still, most of these strategies turn out to be costly with low efficiency (Yenish 2007). The pre-emergence application of broad-spectrum herbicides such as metribuzin and imazethapyr is regarded as one of the most effective and economical methods to control weeds in lentil fields because of their ability to suppress weed growth and prevent yield losses (Elkoca *et al.* 2005). These pre-emergence herbicides control weeds at the early stage of crop growth, but weeds germinating after crop emergence become a menace to crop production (Gaur *et al.* 2013). Indeed, lentil cultivars are highly sensitive to these herbicides when used as post-emergence treatment. Metribuzin ($C_8H_{14}N_4OS$), which belongs to the triazinone family is a pre- and post-emergence herbicide used to control both broadleaf and grass weeds in crops like soybeans (Soltani *et al.* 2005). Imazethapyr, an imidazolinone herbicide, can be used as a pre-and post-emergence herbicide to effectively control a wide range of weeds in legume crops, especially lentils (Hanson and Thill 2001; Teja *et al.* 2017). Therefore, selection of germplasm tolerant to post-emergence herbicides would be one of the major strategies to control weeds in lentils. Selection for combined resistance to both herbicides with different modes of action would allow herbicide rotation to retard the selection of herbicide resistance in weeds.

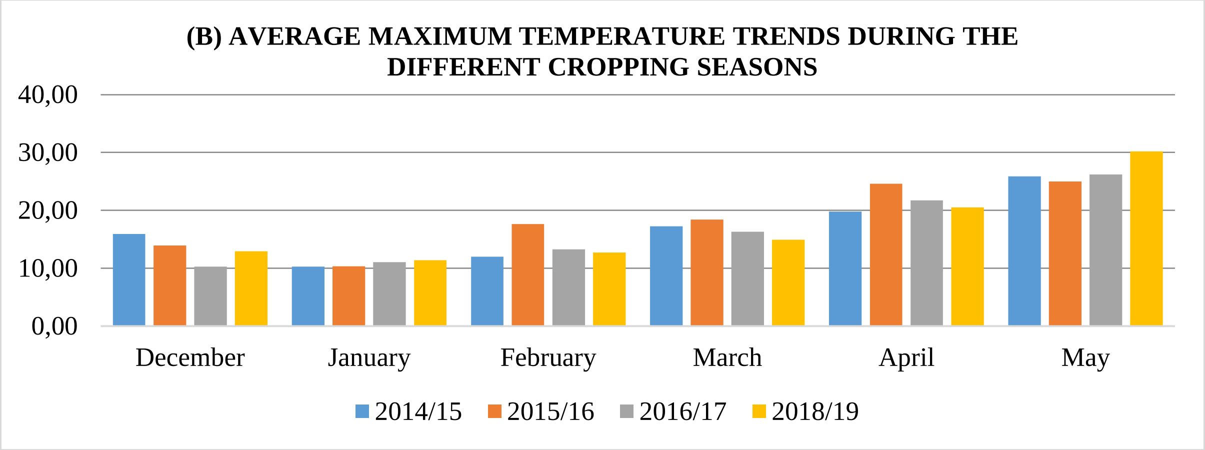
Several studies were conducted in lentils to identify tolerance to herbicides (Hanson and Thill 2001; Fedoruk *et al.* 2011; Yadav *et al.* 2013). In Australia, metribuzin tolerance in lentils has been identified (Mcmurray 2019) as means of enabling the control of broadleaf weeds. In West Asia and North Africa there is a need to develop herbicide tolerant lentils to control weeds such as *Orobanche crenata* and *Cuscuta* ssp. and other annual broadleaves. Therefore, the present study was undertaken in the region to identify promising lentil accessions tolerant to both metribuzin and imazethapyr and to assess the efficiency of adopted herbicide tolerance scores. The second objective was to evaluate the performance and stability of selected accessions under diverse environments.

2. Materials and Methods

2.1.Materials and experiments

221 accessions of cultivated lentils, among them 105 landraces collected from 38 countries and 116 breeding lines developed at the International Center for Agricultural Research in the Dry Areas (ICARDA), were evaluated for their response to imazethapyr and metribuzin applied at the post-emergence stage under field conditions. The experiments were conducted at ICARDA experimental research station, Terbol-Lebanon (33.81° N, 35.98° E) at 890 meters above sea level. Terbol is characterized by cool winter and high rainfall as typical of its continental to semi-arid climate with clay soil. The average precipitation during the crop seasons was 537 mm and the average temperature fluctuated between -1°C and 31°C (Fig. 1).





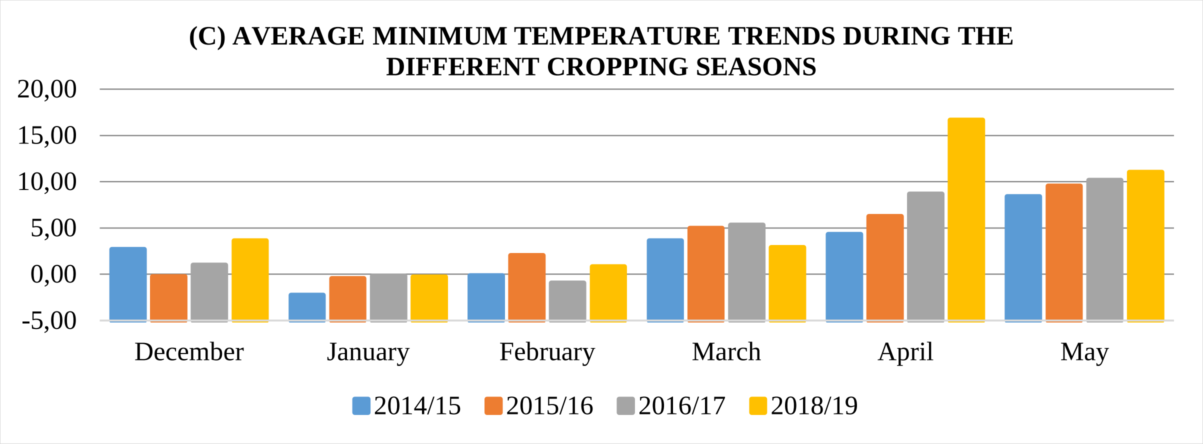


Figure 1. (A): Precipitation (mm) trends during the cropping seasons of the screened years. (B): Variation of maximum temperature (˚C) during the different cropping seasons. (C): Minimum temperature (˚C) during the different cropping seasons.

In this study, we conducted four experiments: two preliminary experiments (Experiment 1 and 2) and two validation experiments (Experiment 3 and 4), where imazethapyr and metribuzin were applied at the pre-flowering stage (5–6th node stage, 10–15 cm plant height). Standard agronomic practices were applied as following: soil fertilization by adding nitrogen, phosphorus and potassium fertiliser (NPK 15-15-15) at 250 kg/ha; weeds were controlled by a pre-emergence application of pendimethalin at 1200 g active ingredient (a.i.)/ha followed by manual weeding to control weeds; sitona was controlled by spraying lambda-cyhalothrin at 40 g a.i./ha; thrips were controlled by a combination of thiamethoxam and acetamiprid at 200 g a.i./ha each and fungal diseases were controlled by a combination of azoxystrobin and difenoconazole respectively at 72.8 and 45.6 g a.i./ha.

The experiments were planted in late November and harvested in late May in rotation with cereals; durum wheat [Triticum turgidum L. subsp. durum (Desf.) van Slageren] or bread wheat (*Triticum aestivum* L.). The plot size of the four experiments was a single row of 1 m length with 0.3 m distance between rows. The details of the experiments are as follows:

Experiment 1 comprised 221 accessions and two repetitive tolerant checks (IG4400 and IG4605; previously identified at ICARDA in preliminary screening); which were conducted in a strip design with two treatments and two replicates during 2014/15. The two treatments imazethapyr at 112.5 g a.i./ha (1.5TI) and metribuzin at 315 g a.i./ha (1.5TM) were applied at 150% of the recommended dose as per the label recommendation of the two herbicides metribuzin (Sencor: Bayer) and imazethapyr (Pursuit: BASF).

Experiment 2 comprised 38 accessions selected from Experiment 1, including 34 tolerant and four susceptible accessions (Table 1); it was conducted in an alpha design with two replicates during 2015/16. Two herbicide treatments, imazethapyr 112.5 g a.i./ha (1.5TI) and metribuzin 315 g a.i./ha (1.5TM), were applied at 150% of the recommended doses.

Experiment 3 The same set of 38 accessions (Table 1) was evaluated for their performance against imazethapyr ($1T_I$: 75 g a.i./ha) and metribuzin ($1T_M$: 210 g a.i./ha) and compared with control (C) during 2016/17.

Experiment 4 The same set of 38 accessions (Table 1) was again assessed for their performance against five treatments, namely imazethapyr (1TI: 75 g a.i./ha) and (1.5TI: 112.5 g a.i./ha), metribuzin (1TM: 210 g a.i./ha) and (1.5TM: 315 g a.i./ha) and control (C) during 2018/19.

Table 1. List of selected lentil accessions evaluated for imazethapyr and metribuzin tolerance in the validation trials during 2016/17 and 2018/19 seasons.

Geno	**IG**	**CROP_NO**	**ORI**
1	IG 262	262	Cyprus
2	IG 323	323	Serbia
3	IG 1878	1878	Turkey
4	IG 2131	2131	Syria
5	IG 2194	2194	Pakistan
6	IG 4400	4400	Syria
7	IG 4605	4605	Argentina
8	IG 4637	4637	Chile
9	IG 5244	5244	Jordan
10	IG 5562	5562	Jordan
11	IG 5628	5628	Spain
12	IG 5722	5722	ICARDA
13	IG 5769	5769	ICARDA
14	IG 70070	6015	ICARDA
15	IG 71379	6447	ICARDA
16	IG 73647	6783	ICARDA
17	IG 75882	7163	Pakistan
18	IG 76266	7547	ICARDA
19	IG 114670	7668	ICARDA
20	IG 114685	7683	ICARDA
21	IG 122889	8077	ICARDA
22	IG 122907	8095	ICARDA
23	IG 122915	8109	Argentina
24	IG 122916	8110	Bulgaria
25	IG 122918	8112	Pakistan
26	IG 122921	8115	ICARDA
27	IG 4152	4152	India
28	IG 4606	4606	Palestine

29	IG 5533	5533	Greece
30	IG 5553	5553	Mexico
31	IG 69577	5968	Cyprus
32	IG 73734	6870	Syria
33	IG 1005	1005	Chile
34	IG 117646	7946	ICARDA
35		8008	ICARDA
36		8009	ICARDA
37	IG 156571	10748	ICARDA
38	LRIL-22-46	LRIL-22-46	ICARDA

2.2.Recorded Observations

Based on the lentil ontology (Kumar and Rajendran 2016), the following observations were recorded:

Herbicide damage score (HDS): HDS was recorded following a 1 to 5 scale after two weeks (HDS1) and five weeks (HDS2) of herbicide application to assess the ability of accessions to recover from the herbicide treatments (Gaur *et al.* 2013). The detailed description of HDS is as following:

HDS= 1: No Damage occurred, no symptoms of phytotoxicity shown and the plants are in excellent shape with a similar appearance to the control plants.

HDS= 2: Slight damage observed by a light inhibition of growth with a marginal yellowing of some leaves; these plants continue normal vegetative growth to flowering and podding stages.

HDS= 3: Moderately damaged accessions showing a clear difference with the untreated plot by the appearance of necrosis on leaves and a lower vegetative growth with a clear deformation on the newly formed apical leaves and a rate of mortality below 25%. Plants at this stage were able to proceed to the flowering and podding stages.

HDS= 4: severely damaged accessions where plants have a poor vegetative growth caused by a severe chlorosis, narrowing, and burning of leaves. These plants stop the development of new leaves totally and the mortality rate varies between 25% and 75%. The flowering stage is heavily affected since the flowers are burned.

HDS= 5: Severe damage of the crop with mortality above 75% per plot.

Crop phenology: Observations were taken on days to 50% flowering (DF) and 95% maturity (DM) on plot basis.

Agronomic and yield traits: At maturity, three plants were taken randomly to record observations on plant height (PH), biological yield/plant (BY), number of pods/plant (NPP), number of seeds/plant (NSP), and yield/plant (SY) and the average of three plants was used for statistical analysis.

Reduction indices: Reduction index (RI_{trait}) was estimated to measure the performance of selected tolerant accessions, as follows (Sharma *et al.* 2018):

$$RI_{trait} = 100 - \frac{(100 \times \overline{\mathrm{T}})}{\overline{\mathrm{C}}}$$

Where ($\overline{\mathrm{T}}$) is the trait value of evaluated accession under herbicide treatments, and $\overline{\mathrm{C}}$ is the value of the same accession under controlled conditions without any herbicide treatments. This reduction index was calculated for plant height (PH), biological yield per plant (BY) and seed yield per plant (SY).

2.3.Statistical Analysis

The statistical row-column model was applied to detect differences among accessions (A), herbicide treatments (T) and their interaction (A x T) for phenological and agronomic traits using the Genstat statistical software (Goedhart and Thissen 2010). Differences in the effects of accessions and herbicide treatments were assessed using p values. The best-unbiased values of each accession and treatment were estimated by the applied statistical software. Ordinal regressions analysis was performed between HDS1 and HDS2 and the reduction index to assess the efficiency of the herbicide tolerance score.

Multiple experiments analysis led over the years was conducted using the method of residual maximum likelihood (REML) where A, T and A x T were fitted in the fixed model while years, replicates and blocks were fitted in the random model. In addition, each herbicide treatment in every season was considered an independent environment to assess the stability of 38 accessions selected for herbicide tolerance in the preliminary studies. Genetics, genetics × environment (GGE) biplot of multi-environment trial (MET) analysis of these accessions were conducted using the best linear unbiased predictions (BLUPs) to evaluate their replicability over the seasons under diverse herbicides. A line was drawn to connect each treatment to the biplot origin to visualize the relationship between the herbicide treatments, called vectors. The angle between two vectors was used to approximate the correlation between the two herbicide treatments (Yan and Tinker 2006; Kaya and Turkoz 2016). The smaller the angle between two vectors, the higher is the correlation between the two environments. Finally, the biplot showed the mega environments by drawing an ellipse around similar environments which share the same sector.

3. Results

3.1.Herbicide damage Score

The HDS1 score of lentil accessions ranged between 2 and 5 for imazethapyr at 112.5 g a.i./ha (1.5TI) during 2014/15, showing wide range of variation among herbicide tolerance. Among 221 accessions tested, 21 accessions scored two with slight damage on leaves with marginal yellowness, 123 accessions scored three with moderate damage with leaf necrosis, 68 accessions with score four were severely damaged with 25–75% mortality, and nine accessions scored five with total mortality. The HDS2 score, taken after 5 weeks of herbicide treatment, indicated accentuated damage in all accessions. Based on the HDS2 score, ten accessions with marginal leaf yellowness recorded scores of two, 92 with moderate levels of damage scored three, 107 accessions with severe damage scored four and finally, 12 accessions with total crop failure scored five. For metribuzin at 315 g a.i./ha treatment (1.5TM), HDS1 showed wide variation with seven accessions scoring two with minimum damage (marginal leaf burning), 85 scoring three with moderate damage (leaf necrosis and lower vegetative growth), 127 scoring four with high damage (severe leaf burning) and two accessions scoring five with total mortality for more than 75% of plants in the treated plot. The HDS2 score, taken after 5 weeks of herbicide treatment, showed recovery from the herbicide damage with the formation of new leaves. The HDS2 score showed that only one accession scored one with no visible damage, 31 scored two with slight damage, 114 scored three with moderate damage, 73 scored four with a mortality rate between 25 and 75% and two accessions scored five with a mortality rate above 75%.

Based on the herbicide damage score in the preliminary screening trials, 38 accessions were selected for further evaluation to confirm their tolerance. Validation trials conducted during 2018/19 (Experiment 4) showed that lentil accessions recovered from the herbicide damage within 5 weeks after the application of imazethapyr at 75 g a.i./ha whereas the damage was accentuated when treated with imazethapyr at 112.5 g a.i./ha (1.5TI) (Fig. 2). On the other hand, the results of Experiment 3 conducted during 2016/17 showed that the damage was accentuated 5 weeks after treatment with imazethapyr at 75 g a.i./ha. For metribuzin, lentil accessions showed recovery from the herbicide damage after 5 weeks of metribuzin treatments during 2016/17 and 2018/19 (Fig. 2).

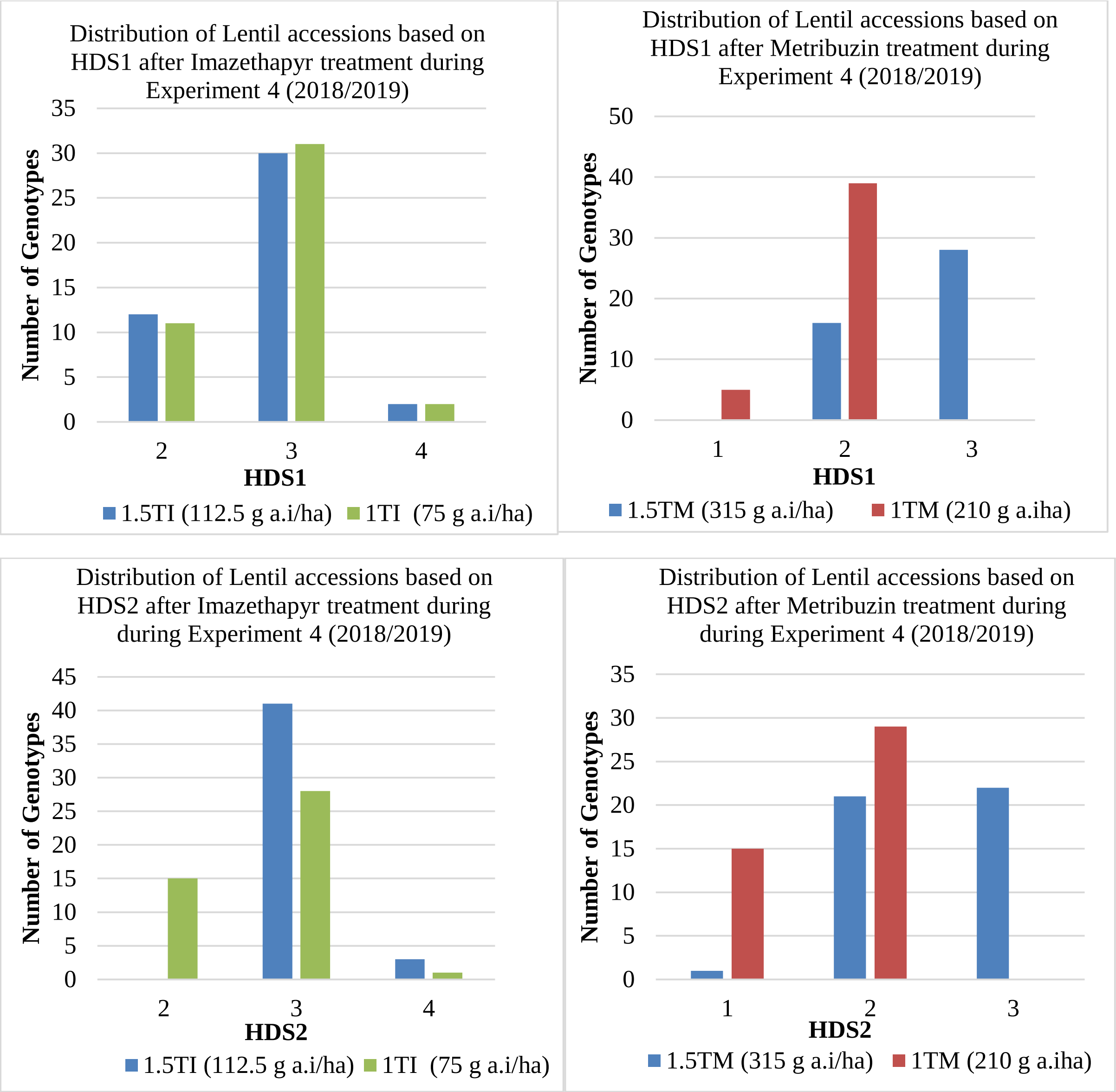


Figure 2. Distribution of lentil accessions along with Herbicide Damage (HDS1 and HDS2) recorded after two and three weeks of treatment with different doses of Imazethapyr and Metribuzin.

3.2.Crop phenology

Combined analysis of variance showed that for days to 50% flowering (DF) and 95% maturity (DM) data, $p < 0.001$ among accessions (A), herbicide treatment (T) and years (Y) was detected (Table 2). Analysis of variance for these traits at each experiment among accessions and herbicide treatments also showed that p was less than 0.001 over the years except for DF among herbicide treatments (T) during 2015/16 (Experiment 2) (Table 3). Moreover, $p < 0.001$ obtained for the A × T interactions over the years except for DF during 2014/15 (Experiment 1).

Table 2. Combined analysis performed for preliminary and advanced screening trials to analyze significance differences (p value) for the studied traits among years, accessions, herbicide treatments and the interaction between year, accessions and herbicide treatments.

Factors	DF (df)	DM (df)	PH (df)	BY (df)	SY (df)	NPP (df)	NSP (df)	RI_{PH} (df)	RI_{BY} (df)	RI_{SY} (df)
Year (Y)	<0.001 (3)	<0.001 (2)	<0.001 (3)	<0.001 (2)	<0.001 (2)	0.145 (1)	<0.001 (1)	<0.001 (1)	0.436 (1)	<0.001 (1)
Accessions (A)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)	<0.001 (37)
Herbicide Treatment (T)	<0.001 (4)	<0.001 (4)	<0.001 (4)	<0.001 (4)	<0.001 (4)	0.007 (4)	<0.001 (4)	0.003 (3)	0.011 (3)	<0.001 (3)
A x T	0.002 (148)	0.615 (148)	0.884 (148)	0.011 (148)	0.968 (148)	0.212 (148)	0.732 (148)	<0.001 (110)	0.098 (111)	<0.001 (111)
Y x A	<0.001 (111)	<0.001 (74)	<0.001 (111)	<0.001 (74)	<0.001 (74)	<0.001 (37)	<0.001 (37)	0.005 (37)	<0.001 (37)	<0.001 (37)
Y x T	0.003 (4)	0.005 (3)	0.025 (4)	0.025 (3)	<0.001 (3)	0.001 (2)	<0.001 (2)	0.028 (1)	0.576 (1)	0.016 (1)
Y x A x T	0.004 (146)	0.393 (108)	0.795 (137)	0.782 (111)	0.505 (111)	0.196 (74)	0.125 (74)	0.168 (33)	0.02 (37)	<0.001 (36)

DF: days to flowering, DM: days to maturity, PH: plant height, BY: biological yield per plant, SY: yield per plant, NPP: number of pods per plant, NSP: number of seeds per plant, RI_{PH}: reduction index of plant height, RI_{BY}: reduction index of biological yield per plant, RI_{SY}: reduction index of yield per plant.

Table 3. Spatial model analysis performed for preliminary and advanced screening trials to analyze significance differences (p value) for the studied traits among accessions, herbicide treatments and the interaction between accessions and herbicide treatments.

Factors	Experiment	df	DF (df)	DM(df)	PH (df)	BY (df)	SY (df)	NPP (df)	NSP (df)	RI_{PH} (df)	RI_{BY} (df)	RI_{SY} (df)
A	Experiment 1	220	<0.001	<0.001	<0.001	<0.001	<0.001	ND	ND	ND	ND	ND
T		1	0.01	0.114	0.314	0.35	0.021	ND	ND	ND	ND	ND
A x T		220	0.362	0.982	0.911	0.001	0.887	ND	ND	ND	ND	ND
A	Experiment 2	38	<0.001	ND	<0.001	ND	ND	ND	ND	ND	ND	ND
T		1	0.604	ND	0.111	ND	ND	ND	ND	ND	ND	ND
A x T		38	<0.001	ND	0.013	ND	ND	ND	ND	ND	ND	ND
A	Experiment 3	43	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T		2	<0.001	<0.001	<0.001	<0.001	<0.001	0.037	0.249	0.069	<0.001	0.036
A x T		83	0.003	0.039	0.273	<0.001	<0.001	0.06	0.057	0.029	<0.001	<0.001
A	Experiment 4	37	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
T		4	<0.001	<0.001	<0.001	0.003	0.012	0.014	0.005	<0.001	0.159	0.001
A x T		149	<0.001	0.448	0.275	0.323	0.477	0.011	0.092	<0.001	0.233	0.003

Experiment1: 2014/2015, Experiment 2: 2015/2016, Experiment 3: 2016/2017, Experiment 4: 2018/2019, Accessions (A), Herbicide Treatment (T), ND: Not Determined, DF: days to flowering, DM: days to maturity, PH: plant height, BY: biological yield per plant, SY: yield per plant, NPP: number of pods per plant, NSP: number of seeds per plant, RI_{PH}: reduction index of plant height, RI_{BY}: reduction index of biological yield per plant, RI_{SY}: reduction index of yield per plant.

It was observed that the pre-flowering phase was prolonged in plots treated with imazethapyr than in metribuzin. Experiment 1 showed that flowering date in lentil accessions was delayed by an average of 4.9 days in the imazethapyr treatment at 112.5 g a.i./ha (1.5TI), when compared to metribuzin treatment at 315 g a.i./ha (1.5TM). In addition, during 2016/17, the average flowering time in lentil accessions was delayed by 7.6 days for imazethapyr at 75 g a.i./ha treatment. In contrast, for metribuzin at 210 g a.i./ha, there was no delay when compared with the control (Table 4). The same results were observed in the 2018/19 season.

Table 4. Means and standard errors of tested traits of lentil accessions in function of different herbicide treatments of the validation trials at Terbol, Lebanon.

Treatment	DF	DM	PH	BY	SY	NPP	NSP	RI_{PH}	RI_{BY}	RI_{SY}
Experiment 3 (2016/2017)										
Imazethapyr (75 g a.i/ha) ($1T_I$)	140.2	172.9	22.3	4.7	0.4	42.1	12	31.4	54.3	84
Metribuzin (210 g a.i/ha) ($1T_M$)	133.4	168.8	24.7	8.9	0.9	62.4	24.7	22.1	31.6	56.4
Control (C)	132.6	167	29.8	16.8	2.5	89.3	54.1			

SE	0.5	1.3	1.5	0.67	0.13	6.8	7.8	ND	2.7	4.4
Experiment 4 (2018/2019)										
Imazethapyr (75 g a.i/ha) ($1T_I$)	152.5	194	29.4	12	1.2	43.4	26.3	26.2	40	72.2
Imazethapyr (112.5 g a.i/ha) ($1.5T_I$)	152.9	194.6	32.1	12.4	1.4	45	30.8	19.4	33.4	75.8
Metribuzin (210 g a.i/ha) ($1T_M$)	143.1	181.8	40.5	14.4	3.2	92.5	77.6	5.4	24.9	30.8
Metribuzin (315 g a.i/ha) ($1.5T_M$)	145	186.6	36.4	15.1	3.1	96.8	76	11.3	23.1	35.5
Control (C)	142.8	183.5	38.2	18.4	4.3	129.5	103.9			
SE	1.5	2.9	1.8	2.03	0.5	13.7	9.4	3.2	7.7	3.6

$1.5T_I$: Imazethapyr 112.5 (g a.i/ha), $1.5T_M$: Metribuzin 315 (g a.i/ha), $1T_I$: Imazethapyr 75 (g a.i/ha), $1T_M$: Metribuzin 210 (g a.i/ha), C: Control, DF days to flowering, DM days to maturity, PH plant height, BY biological yield per plant, SY: yield per plant, NPP: number of pods per plant, NSP: number of seeds per plant, RI_{PH}: reduction index of plant height, RI_{BY}: reduction index of biological yield per plant, RI_{SY}: reduction index of yield per plant.

The number of days to maturity of lentil accessions was prolonged in plots treated with imazethapyr (1TI), but not in plots treated with metribuzin (1TM) during 2016/17 and 2018/19. Moreover, when increasing the dose of both herbicides, no delays in either flowering and maturity time were observed during 2018/19 (Table 4).

3.3.Agronomic Traits

Combined analysis of variance showed that for plant height, *p* was less than 0.001 among accessions (A) and herbicide treatments (T) (Table 2). In each experiment, we observed that *p* was less than 0.001 for plant height among lentil accessions (A) and herbicide treatments (T) over the years except for the herbicide treatments (T) during 2014/15 and 2015/16 (Table 3). The average plant height of lentil accessions was shorter under herbicide treatments than in untreated control plots except when compared with metribuzin treatments (1TM and 1.5TM) in 2018/19 (Table 4).

3.4.Yield attributes

Combined analysis of variance showed that for biological yield (BY), $p < 0.01$ was observed among accessions (A) and herbicide treatments (T) (Table 2). At the level of each experiment, we observed that *p* was inferior to 0.001 for biological yield among lentil accessions (A) and herbicide treatments (T) except for treatments (T) during 2014/15 (Table 3). During 2016/17, the average biological yield of the untreated control plots (control) was higher than the average biological yield of imazethapyr at 75 g a.i./ha (1TI) and metribuzin at 210 g a.i./ha (1TM). However, during 2018/19 (Experiment 4), the average biological yield of the untreated control plots was higher than the average biological yield of lentils treated with imazethapyr with any dose but showed no difference with either dosage of metribuzin (Table 4). Combined analysis of variance showed that p was less than 0.01 for seed yield (SY), number of pods per plant (NPP) and number of seeds per plant (NSP) among accessions (A) and herbicide treatments (T) (Table 2). In each experiment, p was inferior than 0.001 for seed yield, number of pods per plant and number of seeds per plant among lentil accessions (A) and herbicide treatments (T) over the years, except for the herbicide treatments (T) of number of pods per plant and number of seeds per plant during 2016/17 and of seed yield and number of pods per plant during 2018/19 (Table 3). The Accessions × Treatment (A × T) interaction showed p value above 0.05 indicating that there is no interaction between accessions and herbicide treatments applied (Table 3). All yield attributes (SY, NPP and NSP) were higher in untreated control plots than the herbicide-treated plots (Table 4). However, increased dosages of herbicides did not further affect the seed yield, number of pods per plant and number of seeds per plant as exemplified in Experiment 4 during 2018/19 (Table 4).

3.5.Reduction index

Combined analysis of variance showed that p was less than 0.01 for reduction index of plant height (RI_{PH}), biological yield per plant (RI_{BY}) and yield per plant (RI_{SY}), among accessions (A) and herbicide treatments (T) (Table 2). At the level of each experiment, the analysis of variance for the reduction index of plant height (RI_{PH}), biological yield per plant (RI_{BY}) and yield per plant (RI_{SY}), $p < 0.005$ among accessions (A), herbicide treatments (T) and A × T interaction during all the cropping seasons except among the herbicide treatments (T) of RI_{PH} during 2016/17 (Experiment 3) and among the herbicide treatments (T) and A × T interaction of RI_{BY} during 2018/19 (Experiment 4) (Table 3).

The ordinal regression analysis of Experiment 3 conducted during 2016/17 showed that for the reduction index of plant height (RI_{PH}), biological yield (RI_{BY}) and seed yield (RI_{SY}), p was less than 0.05 with the herbicide damage score (HDS2) (Table 5). The average RI_{PH}, RI_{BY} and RI_{SY} increased from 27.8 to 39.8%, from 46.5 to 98.3% and from 78.6 to 99.6% when the herbicide damage score (HDS2) increased after the treatment of imazethapyr with 75 g a.i./ha during 2016/17. The same observation was made for metribuzin. The results of Experiment 4 conducted during 2018/19 indicated no ordinal regression between HDS2 score and reduction indexes for biological yield (BY) and seed yield (Table 5).

Table 5. Ordinal regression (expressed in *p* value) estimate regression parameter and best linear unbiased phenotype values of reduction Index (%) of plant height (RI_{PH}), biological yield per plant (RI_{BY}) and grain yield per plant (RI_{SY}) for different levels of herbicide damage in each treatment.

HDS2	Treatment	RI_{PH} (%)	RI_{BY} (%)	RI_{SY} (%)
		Experiment 3		
1	Imazethapyr (75 g ai/ha)	ND	ND	ND
2		27.8	46.47	78.61
3		29.8	49.7	85.08
4		39.8	58.37	91.72
5		ND	98.32	99.57
Regression (p value)		<0.05	<0.01	<0.01
Estimate parameter ($*10^{-3}$)		41.3*	28.5**	57.6**
1	Metribuzin (210 g ai/ha)	ND	ND	ND
2		18.9	48.84	15.14
3		27.69	59.51	38.04
4		29.81	60.37	63.93
5		ND	90.25	79.11
Regression (*p* value)		<0.05	<0.001	<0.01
Estimate parameter ($*10^{-3}$)		43.2*	51.2***	22.28**
		Experiment 4		
1	Imazethapyr (112.5 g ai/ha)		ND	ND
2			27.72	78.11
3			44.07	72.79
4			51.03	75.89
5			ND	ND
Regression (*p* value)			0.05	0.84
Estimate parameter ($*10^{-3}$)			17.55	-2.3
1	Metribuzin (315 g ai/ha)		35.49	38.19
2			30.63	44.87
3			34.67	41.82
4			ND	ND
5			ND	ND
Regression (*p* value)			0.32	0.76

Estimate parameter (*10^{-3})	14.7	-2.86

HDS2 Second herbicide damage score, ND: Not Determined, RI_{PH}: reduction index of plant height, RI_{BY}: reduction index of biological yield per plant, RI_{SY}: reduction index of yield per plant; $p<0.05$; **$p<0.01$; ***$p<0.001$.

3.6.Selection of tolerant accessions

Correlation analysis between HDS2 and reduction index of plant height (RI_{PH}), biological yield (RI_{BY}) and seed yield (RI_{SY}) showed no correlation between HDS2 of four herbicide treatments (1TI, 1.5TI, 1TM and 1.5TM) and the estimated reduction indexes of three traits (Table 5). The HDS score was helpful in preliminary screening; however, there is a need to rely on the yield and reduction index for validation. In addition, some accessions showed phenological recovery and vegetative growth after treatment, but none was capable of full recovery because of unfavorable environmental conditions. Based on the estimated RI_{SY}, four accessions confirmed their tolerance to both herbicides (IG323, IG5722, IG4400, IG4605) (Table 6). The selected accessions (IG323, IG5722, IG4605, IG4400) did not show a delay in flowering and maturity under different metribuzin treatments (1TM: 210 g a.i./ha and 1.5TM: 315 g a.i./ha). Interestingly, IG4605 flowered earlier than the control even under imazethapyr treatments (1TI) and (1.5TI). Overall, the phenology of tolerant accessions was not affected when treated with any of the herbicides.

Table 6. Herbicide damage scores, plant height, grain yield per plant and reduction indexes of the grain yield and the plant height of the selected accessions and their origin at Terbol 2018/2019 under imazethapyr (75 g a.i/ha) and (112.5 g a.i/ha) and metribuzin (210 g a.i/ha) and (315 g a.i/ha) treatments.

Treatment	HDS1	HDS2	DF	RI_{DF}	DM	RI_{DM}	PH	RI_{PH}	SY	RI_{SY}
					IG323 (SRB)					
$1T_I$ (75 g a.i/ha)	2	2	161.7	11.7	194.8	5.1	28.5	15.5	0.9	31.6
$1.5T_I$ (112.5 g a.i/ha)	3	3	150.7	4.1	188.3	1.6	33.5	2	1.6	24
$1T_M$ (210 g a.i/ha)	2	2	160.7	11.1	192.8	4	38	22.5	4.4	-8.4
$1.5T_M$ (315 g a.i/ha)	2	2	150.7	4.1	186.3	0.5	33	17.5	2.7	25.1
					IG5722					
$1T_I$ (75 g a.i/ha)	3	3	151.8	1.3	197	3.1	27	29.4	1.7	41.5
$1.5T_I$ (112.5 g a.i/ha)	3	3	154.8	3.3	187.5	-1.8	31.5	18.2	2.2	27.2
$1T_M$ (210 g a.i/ha)	2	1	165.8	10.7	197.7	3.5	42	-9.2	2.8	4.1
$1.5T_M$ (315 g a.i/ha)	2	2	153.8	2.7	185.5	-2.9	37	3.9	3.1	-5.5
					IG4605 (ARG)					
$1T_I$ (75 g a.i/ha)	2	2	136.9	0.7	192.8	6.3	34	27.7	2.2	-7.9

1.5T_I (112.5 g a.i/ha)	2	2	132.4	-2.6	184.8	1.9	26.5	-32.5	0.8	2.8
1T_M (210 g a.i/ha)	2	2	132.3	-2.6	192	5.9	40	-4.8	3.4	-2
1.5T_M (315 g a.i/ha)	3	3	132.7	-2.4	178.1	-1.8	40	14.9	2.9	5.5
IG4400 (SYR)										
1T_I (75 g a.i/ha)	3	3	155	8.4	195	8.3	27.5	5.1	4.1	50.3
1.5T_I (112.5 g a.i/ha)	3	3	146	2.1	185.5	3.1	29.5	23.1	1.2	76.6
1T_M (210 g a.i/ha)	2	1	150	4.9	194	7.8	47	-23.1	5.5	24.6
1.5T_M (315 g a.i/ha)	2	2	143	0	178.5	-0.8	40	5.1	4.2	45.7
Terbol 2018/2019 (Experiment 4)										
Standard error (A)			5.67	10.9	1.4	21.7				
Standard error (T)			5.6	10.8	1.4	22.13				
p value (A)			<0.001	<0.001	<0.001	<0.001				
p value (T)			<0.001	<0.001	<0.05	<0.01				
p value (A x T)			0.275	<0.001	0.477	<0.01				

ARG: Argentina, SRB: Serbia, SYR: Syria, **1T_I**: Imazethapyr (75 g a.i/ha), **1.5T_I**: Imazethapyr (112.5 g a.i/ha), **1T_M**: Metribuzin (210 g a.i/ha), **1.5T_M**: Metribuzin (315 g a.i/ha), HDS1: Herbicide damage score 1, HDS2: Herbicide damage score 2, PH: plant height, RI_{PH}: reduction index of plant height, SY: Yield per plant, RI_{SY}: reduction index of yield per plant; (A) between accessions; (T) between treatments; (A x T) interaction between accessions and treatments.

3.7.Replicability Analysis

The tested accessions under validation trials performed differently among seasons and herbicide treatments as *p* was less than 0.001 of Accession × Year × Treatment, and Accession × Year and Treatment × Year (Table 2). GGE biplot analysis was conducted for grain yield to assess the replicability of lentil accessions selected previously in the preliminary studies of herbicide tolerance. The biplot accounted for >70% of the variation in grain yield in relation to genotypes and their interactions with environment (Fig. 3). The biplot was divided into eight sectors and four megaenvironments. Two mega-environments were represented by one season-treatment each: Mega-environment 1 (E1: season 2016/17 of imazethapyr at 75 g a.i./ha) and Megaenvironment 2 (E5: season 2018/19 of metribuzin at 210 g a.i./ha) and the other two representing more than one season-treatment and Mega-environment 3 (E2: season 2016/17 of metribuzin at 210 g a.i./ha and E3: season 2016/17 control untreated with herbicide) and Megaenvironment 4 (E4: season 2018/19 of imazethapyr at 75 g a.i./ha, E6: 2018/19 season of metribuzin at 315 g a.i./ha and E7: 2018/19 season control untreated with herbicide). The two major mega-environments (3 and 4) aligned with the weather conditions during 2016/17 and 2018/19, respectively (Fig. 3).

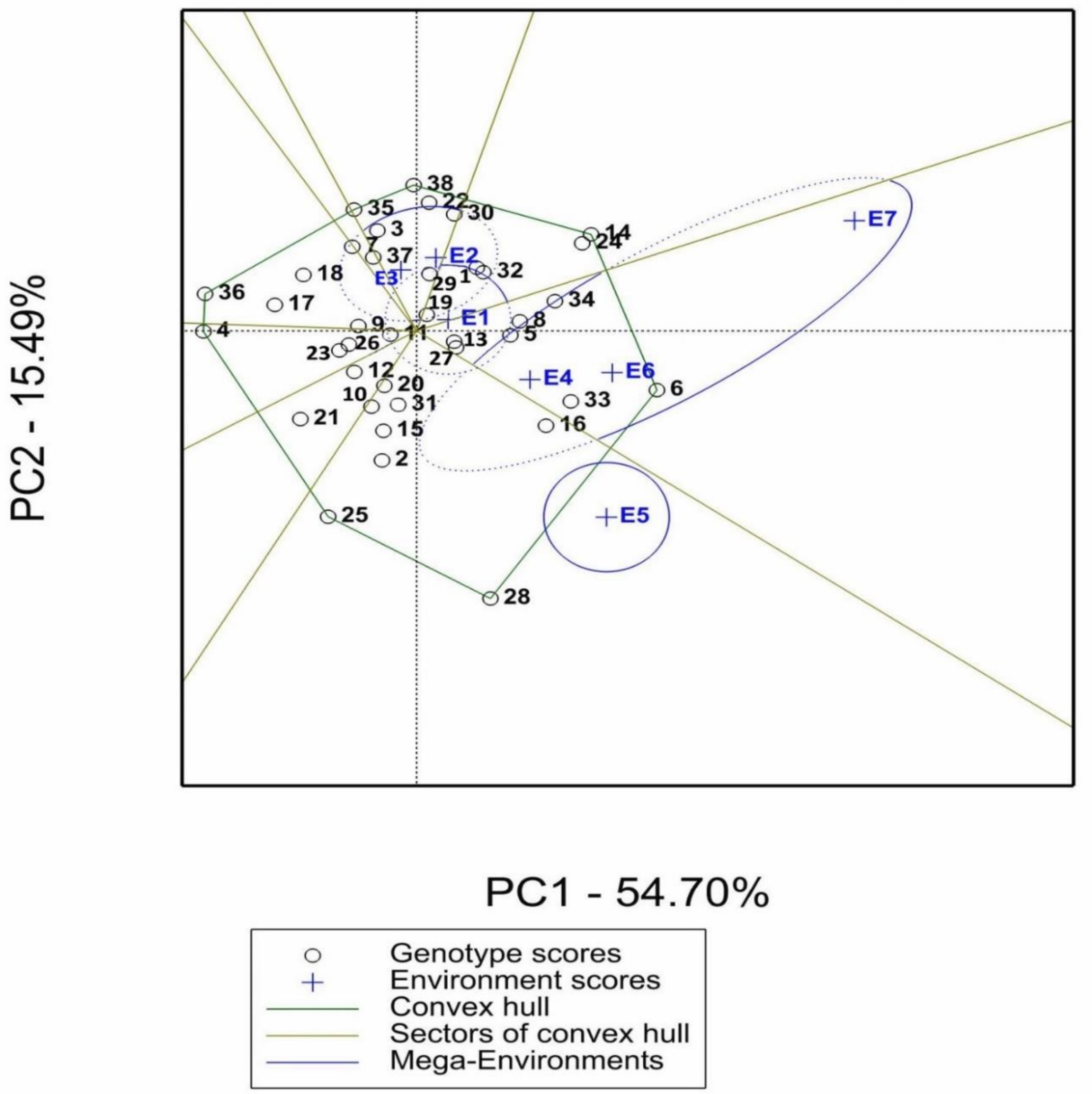


Figure 3. GGE biplot of tested accessions in validation trials for yield data (SY) explained 70.19% of total variability). E1: season 2016/17 of Imazethapyr at 75 g a.i./ha, E2: season 2016/17 of Metribuzin at 210 g a.i./ha, E3: season 2016/17 control untreated with herbicide, E4: season 2018/19 of Imazethapyr at 75 g a.i./ha, E5: season 2018/19 of Metribuzin at 210 g a.i./ha, E6: 2018/19 season of Metribuzin at 315 g a.i./ha, E7: 2018/19 season control untreated with herbicide. Accessions numbered 1 to 38 were listed in Table 1.

Four lentil accessions, namely IG5628, IG5769, IG114670 and IG4152 were located close to the origin, indicating their wide adaptation and highest stability with similar performance under normal growth conditions without herbicide treatments and with either metribuzin and imazethapyr in Mega-environment 1; (E1); and Mega-environment 3; (E2, E3) (Fig. 3). This observation shows the replicability of these accessions in seasons with low rainfall conditions, represented by the environmental conditions of 2016/17, highlighted by a total precipitation equivalent to 458 mm (Fig. 1a).

Six accessions, namely IG2194, IG4637, IG73647, IG1005, IG117646 and IG4400 were located in Mega-environment 4 with no herbicide treatments (control) and were adapted to environments treated by imazethapyr and metribuzin, indicated their wide adaptability with similar performance under the different herbicide treatments. E4, E6 and E7 are 2018/19 environments highlighted by a total precipitation equivalent to 709 mm (Fig. 1a). This shows that the environmental conditions had a higher effect on the accessions than the treatments applied. Fig. 3 also shows that IG4400 is the most tolerant under Mega-environment 4, thus under high rainfall conditions.

The four selected tolerant genotypes showed adaptability to different seasons and herbicide treatments. IG 4400 was located in Mega-environment 4 with no herbicide treatments (control) and was adapted to environments treated by imazethapyr and metribuzin, indicated its adaptability to high rainfall conditions. IG4605 was located in Mega-environment 3 (E2, E3) with no herbicide treatments (control) and was adapted to environments treated by imazethapyr and metribuzin, indicating their adaptability to low rainfall conditions. IG323 is not stable and not adapted to any mega-environment. However, it is located in the same sector of E4 and E5 (E4: season 2018/19 of imazethapyr at 75 g a.i./ha, E5: season 2018/19 of metribuzin at 210 g a.i./ha). Still, the yield data (SY) showed that IG323 had low yield under low rainfall conditions and high yield under high rainfall conditions. Thus, it is a tolerant accession under a high rainfall environment. IG 5722 is located near the center, and in the sector of E1: season 2018/19 of imazethapyr at 75 g a.i./ha. Thus, it is a tolerant accession under a low rainfall environment.

4. Discussion

Weeds are considered a major constraint affecting the production of lentil and the application of herbicides is considered one of the most efficient techniques to control weeds and avoid yield losses in many crops (Garcia De Arevalo *et al.* 1992). In lentil, pre-emergence herbicides are available for use to control weeds efficiently at the early growing stage but not in the North and East Africa regions. Therefore, the selection of lentil accessions tolerant to herbicides is essential to integrate lentil into the cropping system. Our results showed a wide range of genetic variability for herbicide tolerance in lentils which allow introgression of the tolerance to wide adapted cultivars. This observation was reported in earlier studies in lentil (Sharma *et al.* 2016), chickpea (*Cicer arietinum* L.) (Taran *et al.* 2010; Gaur *et al.* 2013; Chaturvedi *et al.* 2014), faba bean (*Vicia faba* L.) (Abou-Khater *et al.* 2021), and field pea (*Pisum sativum* L.) (Hanson and Thill 2001).

4.1.Crop response after herbicide treatments

The herbicide damage in legume crops can be accentuated in susceptible accessions while tolerant ones can recover after being affected the first two weeks of treatments. This has been reported in various studies in lentil (Sharma *et al.* 2018), chickpea (Goud *et al.* 2013), soybean (Belfry *et al.* 2015) and faba bean (Abou-Khater *et al.* 2021). Our experiments showed similar results, after five weeks of herbicide treatments with imazethapyr, the tolerant accessions recovered from the damage while susceptible ones showed accentuated damage. This is expected since lentil is sensitive at less than 5% of the recommended dose of Imidazolinone herbicides (Stork 1995). On the contrary, tolerant accessions could recover from the damage after five weeks even when an increased dose of metribuzin (1.5x) was applied in our experiments. The recovery of vegetative growth can be attributed to the metabolic degradative ability of each accession to metabolize the herbicidal toxic components and detoxify the plants (Shoup *et al.* 2003).

Herbicide damage score was found to be associated with reduction indexes in the experiment conducted in 2016/17, which was a moderately rainy growing season. This indicates the efficiency of the HDS for screening for herbicide tolerance in lentils and aligns with the results obtained in faba bean (Abou-Khater et al., 2021b). However, this observation was not replicated during 2018/19 in our experiments; this is because of heavy rain during the winter season and abnormally high temperatures during pod set (April) and grain filling (May), which affected the growth habit of the plants. For that reason, the reduction index did not correlate with HDS in 2018/2019.

In conclusion, the herbicide damage scores (HDS) gives a general overview about the reaction of genotypes against a tested herbicide. In the case of screening a large number of genotypes, the HDS score can indicate highly susceptible lines, which can be excluded for further testing. In validation trials with a limited number of test entries, reduction indices might be more reliable for validating the reaction of tolerant lines. Therefore, the validation of tolerant accessions in our study was based on the reduction index for yield (RI_{SY}). The same selection method was also adopted in faba bean (Abou-Khater *et al.* 2021). However, Taran *et al.* (2013), and in chickpea and Sharma *et al.* (2018) in lentils found a high correlation between herbicide tolerance and morpho-physiological traits, yield and yield components. Therefore, they relied on the herbicide damage score to rank the tolerance of the tested genotypes. Our study shows that visual assessment of the plant through the HDS after herbicide application is proved to be a rapid and easy method for identifying tolerance to imazethapyr and metribuzin at preliminary trial but not sufficient and reliable in validating highly tolerant lines.

4.2.Effect of herbicides on crop phenology

In all the four experiments conducted, flowering of lentil accessions was delayed under herbicides treatments. Consequently, the pre-flowering phase in treated plots was prolonged. Similar results were also obtained in previous studies in lentils (Sharma *et al.* 2016, 2018), chickpea (Taran *et al.* 2010; Gaur *et al.* 2013; Chaturvedi *et al.* 2014) and faba bean (Abou-Khater *et al.* 2021). This might be explained by the fact that the recovered accessions from herbicide treatments involved secondary plant growth with delayed flowering time and pod setting.

4.3.Effect of herbicides on agronomic and yield traits

Application of imazethapyr and metribuzin reduced plant height and biological yield. Similar observations were made earlier on lentil (Sharma *et al.* 2016, 2018), faba bean (Abou-Khater *et al.* 2021) and chickpea (Taran *et al.* 2010; Goud *et al.* 2013). In addition, yield components reduced in all the conducted experiments. These observations are in agreement with the results of Gaur *et al.* (2013) and Goud *et al.* (2013). Similarly, in lentils, Sharma *et al.* (2016, 2018) reported a reduction in yield and yield attributes in the herbicide-treated accessions when compared to untreated control.

4.4.Replicability

The GGE biplot permits to determine specific and wide adaptability of different accessions to mega environments. Our study indicated two major mega environments as each of them consistently represents various trials (Yan *et al.* 2007), where four selected accessions (IG323. IG4400, IG4605 and IG 5722) showed stability to environments. However, two accessions (IG4400 and IG323) were found to be specifically adapted to herbicide treatments under high rainfall conditions and the other two (IG4605 and IG5722) were found in highly adapted environments characterized by herbicide treatments and low rainfall. This is the first report of the stability of these herbicide tolerant accessions. The accessions were screened at Terbol station assuming that this station represents ideal environments for screening for herbicide tolerance as observed in faba bean (Abou-Khater *et al.* 2021b) . Further investigation is also ongoing to validate the observation made on faba bean by Abou-Khater *et al.* (2021b) and to identify suitable environments for screening for herbicide tolerance in lentil. The selected accessions should be crossed to widen their adaptability to different environments.

5. Conclusion

This study suggests the presence of tolerance to post-emergence herbicide treatments in lentils. An herbicide-tolerant variety is an integral part of the integrated weed management package for the most effective and economical approach for weed management. A large genetic variability for herbicide tolerance was observed in lentil germplasm in our study. Thirteen tolerant genotypes of lentil without any effect of herbicide treatment on phenology were identified. Further investigation is required to study the genetics of herbicide tolerance in these genotypes before their efficient use as a donor in the lentil breeding program. .

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Chapter III

Assessing the Stability of Herbicide-Tolerant Lentil Accessions (*Lens culinaris* Medik.) under Diverse Environments

1. Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest annual crops in the world, as old as the domestication of einkorn, emmer, barley, and pea for cultivation. It originated near south eastern Turkey and Syria around 7500 BC and spread over the near east, Egypt, Central and Southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and eventually to Latin America (Cokkizgin and Shtaya 2013). It is a cool-season legume crop that plays a major role in human nutritional security due to its high protein content (20%–36%), carbohydrate (60%–67%), lipid (<4%), and ash (2%–3%) on a dry basis (Johnson *et al.* 2020)(Johnson *et al.* 2020), in animal feeding, and in soil health and is an essential component for crop rotation, particularly with cereals (Erskine *et al.* 2011). Currently, Canada (39.2%), India (19.4%), Australia (6.5%), Turkey (6.5%), the United States of America (5.0%), Nepal (4.0%), China (2.6%), and Ethiopia (2.4%) are the leading producing countries of lentils (FAOSTAT 2023).

Besides the importance stated above, there is a need to increase productivity of lentil in many countries where it is subjected to severe biotic and abiotic stresses. Weeds are one of the most damaging biotic stresses to lentil productivity causing severe yield losses of up to 95% in North Africa and Western and Central Asia (Rubiales and Fernández Aparicio 2012; Sharma *et al.* 2018). Weeds compete with lentils throughout their life cycle due to their shallow roots, poor early vigor, and slow vegetative growth especially during the cool season (Balech *et al.* 2022). Pre-emergence herbicides are effective in controlling weeds early in crop growth, but weeds germinating after crop emergence at the pre-flowering stage pose a threat to lentil production (Gaur *et al.* 2013b). Weeds can be controlled using mechanical and manual weeding, soil sterilization, and high seed rate density (Redlick *et al.* 2017); but these methods are either inefficient or very expensive (Yenish 2007). Post-emergence herbicides like imazethapyr and metribuzin are effective at controlling weeds in many legume crops, including lentil (Sharma *et al.* 2017b, 2018), chickpea (Gaur *et al.* 2013b), and soybean (Oliveira *et al.* 2017). These herbicides can control broad spectrum of annual and parasitic weeds (Johnson *et al.* 1998) but they are phytotoxic to existing lentil genotypes. Therefore, it is necessary to develop herbicide-tolerant lentil cultivars with stable yield under a variety of conditions (Slinkard *et al.* 2007; Sharma *et al.* 2017b, 2018; Balech *et al.* 2022). However, the wide adaptability of these accessions must be proven in order to include them in commercial farming at a large scale (Turk and Kendal 2017). Therefore, they must be tested under different environments to assess their yield stability.

The performance of a genotype depends on genotypic value (G), environmental effect (E) and GE interaction. Yan et al. (2000) suggested G and GE effects instead of only GE interaction for yield stability analysis. The ranking of different genotypes defines GE interaction under various environmental conditions (Sayar *et al.* 2013) by measuring its plasticity. Additionally, it identifies the most suitable test environments, allocates resources within a breeding program and assists with the selection of germplasm and breeding strategy (De Leon *et al.* 2016).

The stability of any genotype suggests that E and GE interaction does not change its ranking and performance. In lentil (Sabaghnia *et al.* 2008), maize (Fan *et al.* 2007) and grass pea (Sayar and Han 2015), several stability analyses have been used to determine if the tested genotypes are stable. GGE biplot allows visualizing the which-won-where pattern and displays the interrelationships among all test environments. This method allows ranking the genotypes based on the yield and stability performance. Since grain yield is the most affected trait in many crops, we focused on evaluating yield of lentil accessions in our study. The stability of herbicide tolerant accessions in faba bean has previously been reported (Abou-Khater *et al.* 2022b).

Consequently, the primary objectives of this study were (1) to evaluate the performance of lentil accessions under imazethapyr and metribuzin treatments, (2) to assess the yield stability of these accessions across a variety of environments, and (3) to identify the ideal environment for selection.

2. Materials and Methods

2.1. Materials and experiments

In eight separate experiments from 2015 to 2019 under three herbicide treatments imazethapyr: 75 (g *a.i.* ha^{-1}); metribuzin: 210 (g *a.i.* ha^{-1}) and without herbicide treatment at two locations; Marchouch, Morocco (33.56°N, 6.69°W) and Terbol, Lebanon (33.81°N, 35.98°E); 42 lentils accessions with varying degrees of tolerance to either imazethapyr or metribuzin were selected (unpublished data) and evaluated again (Table 6). Each experiment represents a unique environment resulting from the interaction of seasons, locations, and herbicide treatments. The validation trials and their environments are described in Table 7 and the weather conditions are shown in Figure 4.

Table 6. List of selected lentil accessions and their tolerance to imazethapyr and metribuzin in validation trials based on a preliminary trial led at Marchouch-2014/15 (unpublished data).

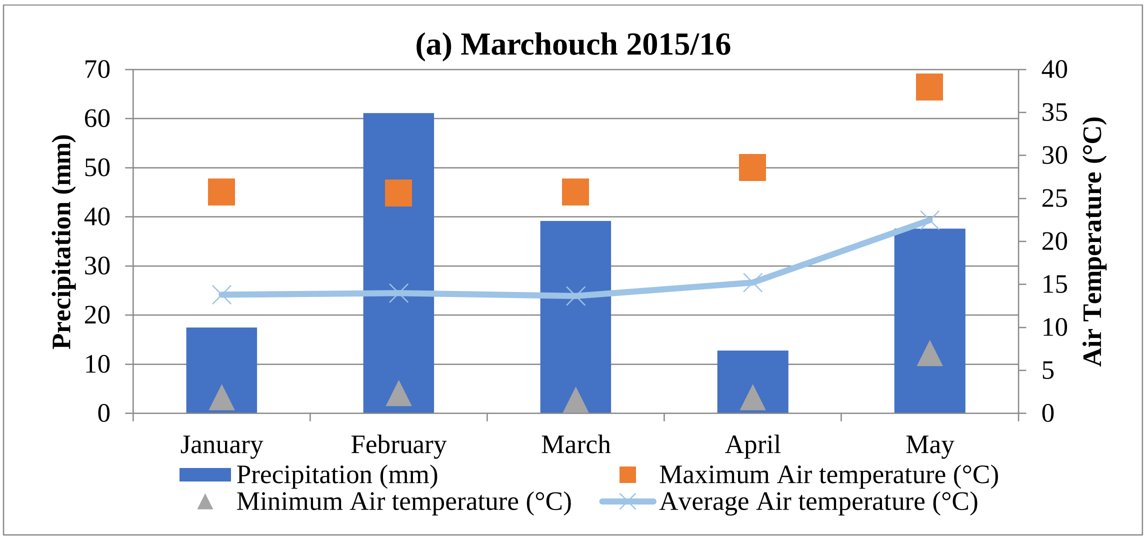
No	Accessions	Tolerance to imazethapyr (75 g *a.i. ha*$^{-1}$)	Tolerance to metribuzin (210 g *a.i. ha*$^{-1}$)
1	IG1455	Moderately Tolerant	Moderately Tolerant
2	IG2445	Moderately Tolerant	Tolerant
3	IG257	Moderately Tolerant	Highly Tolerant
4	IG918	Moderately Tolerant	Tolerant
5	IG5626	Moderately Tolerant	Tolerant
6	IG195	Moderately Tolerant	Highly Tolerant
7	IG462	Moderately Tolerant	Highly Tolerant
8	IG590	Moderately Tolerant	Tolerant
9	IG857	Moderately Tolerant	Tolerant
10	IG156514	Moderately Tolerant	Tolerant
11	IG156633	Moderately Tolerant	Moderately Tolerant
12	IG156635	Moderately Tolerant	Tolerant
13	IG156648	Moderately Tolerant	Tolerant
14	IG156656	Moderately Tolerant	Tolerant
15	IG156771	Moderately Tolerant	Tolerant
16	IG2684	Moderately Tolerant	Moderately Tolerant
17	IG4400	Moderately Tolerant	Tolerant
18	IG4401	Moderately Tolerant	Tolerant
19	IG4605	Moderately Susceptible	Tolerant
20	IG5244	Moderately Tolerant	Tolerant
21	IG5562	Moderately Tolerant	Moderately Tolerant
22	IG5588	Moderately Tolerant	Tolerant
23	IG69492	Moderately Tolerant	Tolerant
24	IG70079	Moderately Tolerant	Tolerant
25	IG71366	Moderately Tolerant	Highly Tolerant

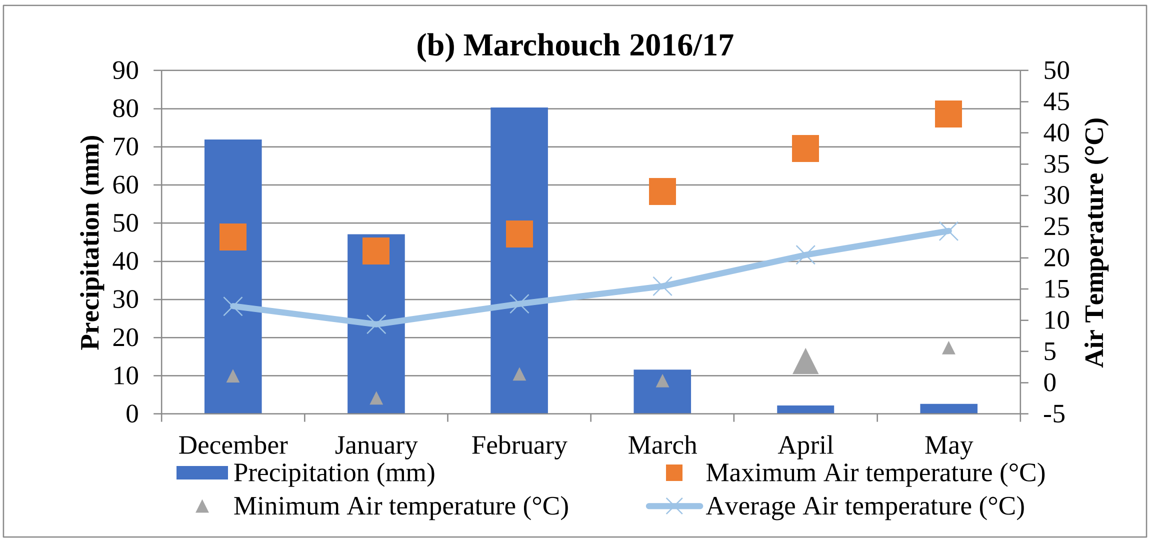
26	IG75929	Moderately Tolerant	Tolerant
27	IG75932	Moderately Tolerant	Tolerant
28	IG76251	Moderately Tolerant	Tolerant
29	IG114663	Moderately Tolerant	Tolerant
30	IG114670	Moderately Tolerant	Tolerant
31	IG114703	Moderately Tolerant	Tolerant
32	IG115370	Moderately Susceptible	Tolerant
33	IG117684	Moderately Tolerant	Tolerant
34	ILL8009	Tolerant	Tolerant
35	IG138106	Moderately Tolerant	Tolerant
36	ILX87075	Moderately Tolerant	Tolerant
37	L24	Moderately Tolerant	Tolerant
38	IG70056	Moderately Tolerant	Tolerant
39	2009S 96568-1	Moderately Tolerant	Tolerant
40	IG156801	Moderately Tolerant	Highly Tolerant
41	010S 96130-1	Moderately Tolerant	Tolerant
42	010S 96155-2	Moderately Tolerant	Tolerant

Table 7. Specifications and details of the various environments tested for lentil screening.

Environment	Environment (Location-Cropping Season-Treatment)	Soil Type	Rainfall (mm)	Air Temperature (°C)		
				AVG	AVG Min	AVG Max
E0	Marchouch-2015/16-metribuzin at 210 g *a.i. ha*$^{-1}$	Vertisols and silty clay	168	18.24	6.71	34.03
E1	Marchouch-2015/16-no herbicide treatment					
E2	Marchouch-2016/17-imazethapyr at 75 g *a.i. ha*$^{-1}$	Vertisols and silty clay	211	14.05	-2.4	42.99
E3	Marchouch-2016/17-metribuzin at 210 g *a.i. ha*$^{-1}$					
E4	Marchouch-2016/17-No Herbicide Treatment					
E5	Terbol-2018/19-imazethapyr at 75 g *a.i. ha*$^{-1}$	Clay loam	810	11.7	-0.28	32.3

E6	Terbol-2018/19-metribuzin at 210 g *a.i. ha*$^{-1}$
E7	Terbol-2018/19-no herbicide treatment


(a) Marchouch 2015/16


(b) Marchouch 2016/17

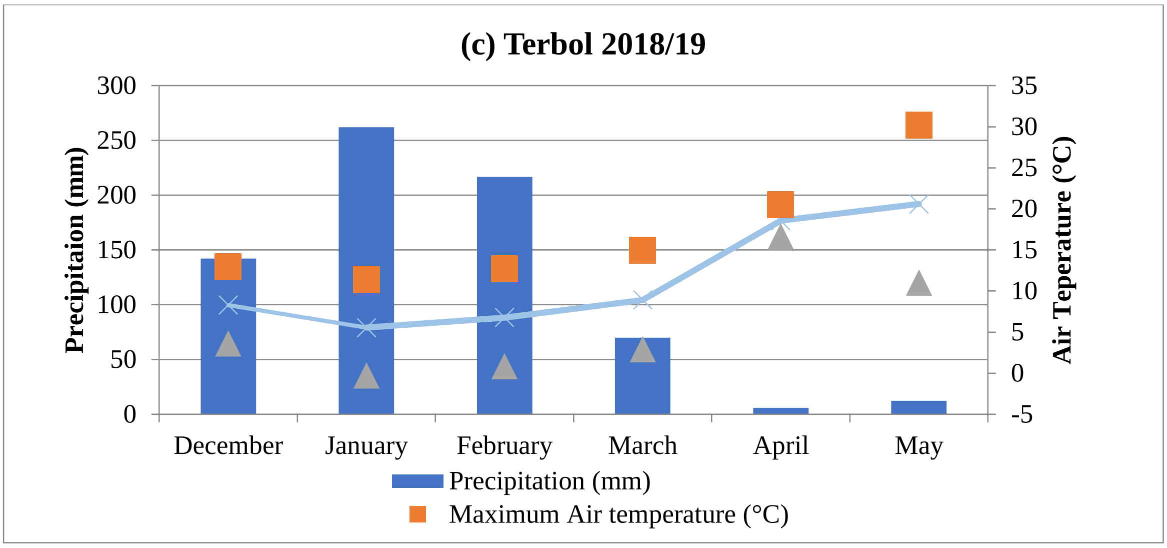


Figure 4. Precipitation (mm), average of minimum and maximum air temperature at Terbol and Marchouch during the three cropping seasons; (a) Marchouch 2015/16; (b) Marchouch 2016/17 and (c) Terbol 2018/19.

The experiments were planted in early December at Terbol and mid-December at Marchouch and both were harvested in late May. The experiment was led out in an alpha lattice design with two replications with a plot size of 1 row, 1(m) length and 0.3(m) width and 40 seeds per plot. Herbicides were applied during the pre-flowering stage (5-6th node stage, 10-15 cm plant height). Except for the post-emergence herbicide treatments, the following agronomic practices were used to raise a successful crop. Trials were conducted in rotation with bread wheat (*Triticum aestivum* L.); soil fertilization with NPK 15-15-15 at 250 kg ha^{-1} applied at pre-sowing stage; pre-emergence application of pendimethalin at 1,200 (g *a.i.* ha^{-1}) followed by three manual weeding from pre-emergence stage till flowering stage to control seasonal weeds; lambda-cyhalothrin at 40 (g *a.i.* ha^{-1}) and a combination of thiamethoxam and acetamiprid at 200 (g *a.i.* ha^{-1}) were applied to control sitona leaf weevil (*Sitona crinitus* Herbst) and thrips (*Frankliniella* spp.); a combination of azoxystrobin and difenoconazole at 73 and 46 (g *a.i.* ha^{-1}) were applied to control fungal diseases especially fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*), and ascochyta blight (*Ascochyta lentis)*.

2.2.Recorded traits

According to Kumar and Rajendran (2016), the lentil ontology was used to identify the following characteristics:

Days to 50% flowering (DF) and 95% maturity data (DM) were crop phenology traits measured from the sowing date. Plant height (PH) (cm), number of pods/plant (NPP), number of seeds/plant (NSP), biological yield per plant in g (BY) and seed yield per plant in g (SY) are agronomical and yield traits that were measured on three plants per plot.

2.3.Statistical methods

2.3.1. Variance analysis

The statistical row-column model was used with Genstat statistical software (Goedhart and Thissen 2018) to assess differences in phenological and agronomic traits among accessions (A) in terms of *p* values using the Wald statistic. The applied statistical software estimated the best-unbiased values of accessions and combined narrow sense heritability (h^2) using the residual maximum likelihood (REML). Differences among accessions were assessed by *p* values using the Wald statistic for each independent environment (E). For DF, DM, BY and SY, the narrow sense heritability values (h^2) were estimated using the residual maximum likelihood method (REML) of Genstat 2019.

2.3.2. Stability parameters

The following five stability parameters were estimated using Genstat statistical software to compare the performance of genotypes across test environments: (1) Cultivar superiority identifies genotypes with superior performance near the maximum in various environments (Lin and Binns 1988); (2) Finlay and Wilkinson parameter identifies lines with general adaptability as those with average stability (bi = 1.0) when associated with high mean yield over tested environment (Finlay and Wilkinson 1963), (3) Shukla parameter identifies the stability of the tested genotypes across different environments (Shukla 1972), (4) Static Stability identifies stable genotypes with stable performance under different environments (Becker and Leon 1988) and (5) Wricke's Ecovalence parameter identifies stability of genotypes based on the GE interaction effects by using the regression approach (Wricke 1962).

2.3.3. GGE biplot

The GGE scatter biplot was constructed using the best linear unbiased phenotypes (BLUPs) of each accession for each environment to determine stability of the seed yield per plant across tested environments. To visualize the relationship between the test environments, a vector line was drawn connecting each environment to the biplot origin. The angle between two vectors was used to approximate the correlation between the environments (Yan and Tinker 2006; Kaya and Turkoz 2016). If the angle between the vectors of two environments is less than 90^o, the two environments are highly correlated. As a result, the smaller the angle between two vectors, the higher the correlation between the two environments. Furthermore, the biplot depicts mega environments by drawing an ellipse around similar environments in the same sector (Turk and Kendal 2017).

The GGE ranking biplot was used to visualize the ranking of accessions based on their SY performance (Yan *et al.* 2000). The ranking biplot abscissa is the line that passes by the biplot origin through the small circle that represents the average of the environments, and its ordinate is the perpendicular line to the abscissa and that passes by its origin. The genotype projections to the abscissa represent the average SY estimates. The parallel projections aid in ranking and testing the variability and stability of genotypes based on their predicted mean yield across environments. The further the projection is far from the axis of the mean environment, the more unstable and variable the genotype under study (Turk and Kendal 2017).

3. Results

3.1. Crop phenology

The combined variance analysis revealed $p < 0.001$ among the 42 accessions (G), across the test environments (E) and their interaction (GE) for days to flowering (DF) and days to maturity (DM) indicating that the genotypes behaved differently under diverse environments. For both traits, the combined narrow sense heritability (h^2) was approximately equal to 0.9 (Table 1).

Table 1. Combined analysis for detecting Wald statistics and differences among genotypes, environments and genotypes x environments interaction (GE) and narrow sense heritability (h^2) for phenological and agronomic traits for the validation trials at Marchouch in 2015/16 and 2016/17 and at Terbol in 2018/19.

Trait	Genotypes (G)			Environment (E)			(GE)			h^2
	d.f.	Wald statistic	*p* value	d.f.	Wald statistic	*p* value	d.f.	Wald statistic	*p* value	
DF	41	862.2	<0.001	8	18556	<0.001	303	904	<0.001	0.93
DM	41	583.5	<0.001	8	17128.9	<0.001	259	418.2	<0.001	0.93
PH	41	125.4	<0.001	5	806.7	<0.001	193	207.6	0.598	0.71
BY	41	337.2	<0.001	8	1995.7	<0.001	298	296.9	0.506	0.21
SY	41	46522.1	<0.001	8	2160.5	<0.001	293	523.9	<0.001	0.57
NSP	41	125.1	<0.001	2	64.1	<0.001	81	114.5	0.01	-
NPP	41	70	0.003	2	55.7	<0.001	81	95.3	0.15	-

G: Genotypes, df: degree of freedom, h^2: narrow sense heritability, DF: days to 50% flowering, DM: days to maturity, PH: plant height, BY: biological yield per plant, SY: seed yield per plant, NPP: number of pods per plant, NSP: number of seeds per plant.

The analysis of variance of DF and DM conducted independently for each environment showed significant differences ($p < 0.001$) among accessions in all environments with the exception of DM at Marchouch-2015/16-metribuzin at 210 (g *a.i.* ha^{-1}) (E0), Marchouch-2015/16-no herbicide treatment (E1), Terbol-2018/19-metribuzin at 210 (g *a.i.* ha^{-1}) (E6), and Terbol-2018/19-no herbicide treatment (E7) (Table 2). For DF, the estimated narrow sense heritability (h^2) ranged from 0.35 in (E0) to 0.87 in (E5), and for DM, it ranged from 0.0 in (E0) and (E1) to 0.7 in (E3) (Table 2).

Table 2. Wald Statistic and *p value* performed estimates for detecting differences across genotypes and narrow sense heritability (h^2) for phenological and agronomic traits.

Environment		DF	DM	BY	SY
E0	Wald statistic	72.8	33.9	65.8	27.7
	p value	0.035	0.407	0.062	0.62
	h^2	0.0	0.0	0.0	0.0
E1	Wald statistic	132.1	36.7	92.4	60.4
	p value	0.014	0.303	6.00E-03	0.115
	h^2	0.4	0.0	0.3	0.0

E2	Wald statistic	112.2	179.1	123.3	203.6
	p value	<0.001	<0.001	0.007	<0.001
	h^2	0.5	0.6	0.5	0.7
E3	Wald statistic	598.4	262.3	81.7	206.0
	p value	<0.001	<0.001	0.05	<0.001
	h^2	0.9	0.7	0.3	0.7
E4	Wald statistic	462.2	93.5	87.8	152.4
	p value	<0.001	0.005	0.042	0.002
	h^2	0.8	0.3	0.3	0.6
E5	Wald statistic	535.9	77.4	66.6	138.4
	p value	<0.001	0.021	0.007	<0.001
	h^2	0.9	0.3	0.2	0.5
E6	Wald statistic	418.9	54.1	56.6	200.4
	p value	<0.001	0.083	0.053	0.03
	h^2	0.7	0.1	0.1	0.5
E7	Wald statistic	152.6	66.6	81.3	153.0
	p value	<0.001	0.062	0.081	0.008
	h^2	0.4	0.2	0.0	0.3

h^2: narrow sense heritability, DF: days to 50% flowering, DM: days to maturity, BY: biological yield per plant, SY: seed yield per plant.

In environments treated with imazethapyr or metribuzin, we found that DF was delayed in all accessions. The earliest flowering was observed in environments (E1, E4 and E7) untreated with herbicides, where the average DF was 99.6, 62.8 and 95.8 days. The widest range of DF among accessions was observed at Terbol-2018/19-imazethapyr at 75 (g *a.i. ha*$^{-1}$) (E5), where DF fluctuated between 90.6 and 118.7 days after sowing (DAS) and the narrowest range of DF was observed at Marchouch-2016/17-imazethapyr at 75 g (g *a.i. ha*$^{-1}$) (E2) (Table 3).

Table 3. Mean ± Standard error (SE) and ranges for different traits in the diverse environments.

Environment		DF	DM	BY (g/plant)	SY (g/plant)
E0	Range	98-118	133-167	0.3-2.4	0.0-0.4
	Mean ± SE	104 ± 0.67	141 ± 2.14	0.9 ± 0.11	0.1 ± 0.01
E1	Range	88-107	141-171	-0.2-6.8	-0.2-1.7
	Mean ± SE	100 ± 0.93	158 ± 4.4	2.4 ± 0.42	0.3 ± 0.21
E2	Range	64-74	96-106	1.6-5.4	0.4-2.1
	Mean ± SE	70 ± 0.17	102 ± 0.61	3.9 ± 0.08	1.1 ± 0.04
E3	Range	52-74	89-106	2.3-6.3	0.6-2.7
	Mean ± SE	63 ± 0.63	98 ± 0.33	4.5 ± 0.12	1.5 ± 0.03
E4	Range	52-73	89-105	2.6-6.4	0.7-2.5
	Mean ± SE	63 ± 0.45	97 ± 1.33	5.1 ± 0.1	1.6 ± 0.04
E5	Range	91-119	134-143	1.1-5.9	-0.0-2.2
	Mean ± SE	104 ± 0.56	139 ± 0.72	3.4 ± 0.37	0.8 ± 0.10

E6	Range	90.3-114	120-140	1.8-7.2	0.4-3.0
	Mean ± SE	97 ± 0.26	129 ± 0.63	3.7 ± 0.25	1.4 ± 0.04
E7	Range	91-107	118-138	1.7-12.6	0.8-4.2
	Mean ± SE	96 ± 1.76	127 ± 1.5	4.8 ± 0.25	2.0 ± 0.13

SE: Standard error, DF: days to 50% flowering, DM: days to maturity, BY: biological yield per plant, SY: seed weight per plant.

Similar to DF, DM was delayed in environments treated with imazethapyr or metribuzin except for Marchouch-2015/16-metribuzin at 210 (g *a.i.* ha^{-1}) (E0) (Figure1 (a, b and c)). The widest range of DM was observed at Marchouch-2015/16 (E0) and (E1) followed by Terbol-2018/19-no herbicide treatment (E7) whereas the range of DM was limited at Terbol-2018/19-imazethapyr at 75 g *a.i.* ha^{-1} (E5) (Table 3).

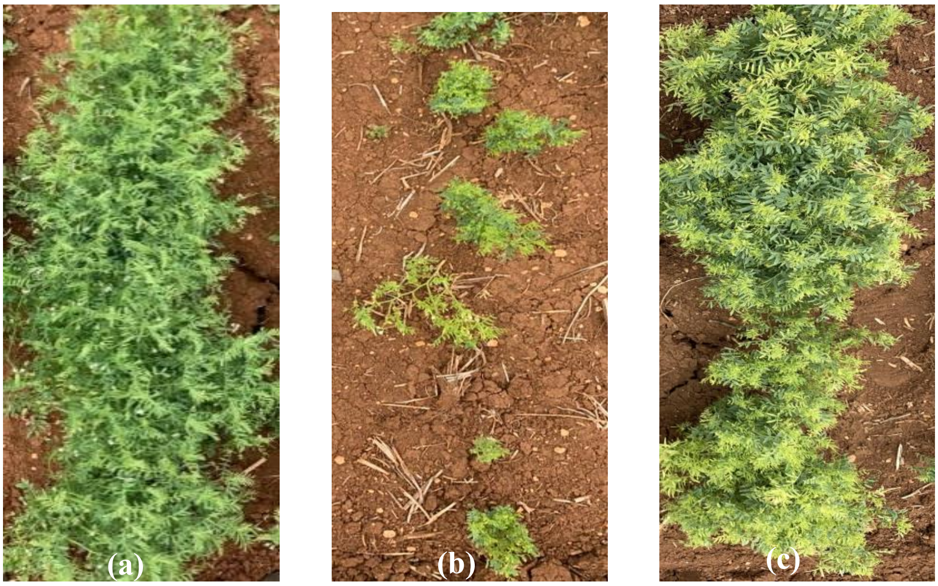


Figure 1. IG115370 (a) plot with no herbicide treatment; (b) plot treated with imazethapyr; (c) plot treated with metribuzin

3.2. Yield attributes

The combined analysis for biological yield per plant (BY), seed yield per plant (SY), number of pods per plant (NPP), and number of seeds per plant (NSP) revealed a significant ($p < 0.001$) variation between genotypes (G), environments (E) and the GE interaction of the eight environments, except for GE interaction of BY ($p = 0.51$) and NPP ($p = 0.15$). This demonstrates that the genotypes of SY and NSP responded differently to various environmental conditions (Table 1).

The average narrow sense heritability (h^2) of BY was 0.21, ranging from zero at Marchouch-2015/16-metribuzin at 210 (g *a.i.* ha^{-1}) (E0) and Terbol-2018/19-no herbicide treatment (E7) to 0.5 at Marchouch-2016/17-imazethapyr at 75 g (g *a.i.* ha^{-1}) (E2) (Table 2). The analysis of variance of BY for each environment revealed that genotypes responded differently to different environments ($p < 0.05$), with the exception of Marchouch-2015/16-metribuzin at 210 (g *a.i.* ha^{-1}) (E0) ($p = 0.062$), Terbol-2018/19-metribuzin at 210 (g *a.i.* ha^{-1}) (E6) ($p = 0.053$) and Terbol-2018/19-no herbicide treatment (E7) ($p = 0.081$) (Table 2). When no herbicide treatment was applied, the average of BY was higher than when imazethapyr or metribuzin were applied. At Marchouch-2016/17-no herbicide treatment (E4), BY was 5.12 g, followed by 4.79 g at Terbol-2018/19-no herbicide treatment (E7) and 2.41g at Marchouch-2015/16-no herbicide treatment (E1). This is then followed by the average BY of environments treated with metribuzin (E3 and E6) and imazethapyr (E2 and E5).

The average narrow sense heritability (h^2) of SY was 0.57, ranging from zero in (E0) and (E1) to 0.72 during Marchouch-2016/17-imazethapyr at 75 (g *a.i.* ha^{-1}) (E2), indicating that each accession responded differently to the various combinations of environments (Table 2). The highest values of narrow sense heritability (h^2) were observed for accessions E2 (0.72), E3 (0.69), E4 (0.58), E5 (0.53), E6 (0.51) and E7 (0.31), indicating repeatability that the trait is replicable among accessions exposed to various herbicides.

The analysis of variance of SY for each environment revealed that genotypes responded differently to various test environments ($p < 0.001$), except for Marchouch-2015/16 (E0 and E1) (Table 2). The average SY at Terbol 2018/19 with no herbicide treatment was the highest (E7), followed by metribuzin 210 (g *a.i.* ha^{-1}) (E6) and then imazethapyr 75 (g *a.i.* ha^{-1}) (E5). Similar observation was made for Marchouch 2016/17 environments (E2, E3 and E4). Comparing the environments treated with imazethapyr (E2 and E5), Marchouch-2016/17-imazethapyr at 75 (g *a.i.* ha^{-1}) had a higher average SY (E2). When comparing the environments treated with metribuzin at Marchouch (E3) and Terbol (E6), the response of genotypes in both environments was identical (Table 3).

3.3. Stability analysis

Significant GE interaction for SY resulted in the estimation of five stability parameters along with their rankings, which are presented in Table 4. At each parameter level, the accessions with the lowest values were considered the most stable. According to the Cultivar superiority index, IG195 is the most yielding and stable line. The most stable accession was ILX87075 based on the static stability index, IG69492 based on Wricke's Ecovalence and Shukla and IG114670 based on Finlay and Wilkinson.

Table 4. Five Stability parameters for seed yield per plant (SY) of selected lentil accessions and their rankings in eight different environments.

Accession number	Accession name	Cultivar Superiority (CS)		Static Stability (SS)		Wricke's Ecovalence (WE)		Shukla (SH)		Finlay and Wilkinson (FW)	
		CS	R_{CS}	SS	R_{SS}	WE	R_{WE}	SH	R_{SH}	FW	R_{FW}
1	IG1455	1.63	40	0.12	4	1.72	30	0.35	35	0.82	2
2	IG2445	1.35	36	0.12	2	1.06	26	0.2	27	1.01	5
3	IG257	1.68	42	0.19	7	0.12	2	0.02	2	0.67	12
4	IG918	0.8	8	0.12	3	0.54	11	0.1	14	1.34	7
5	IG5626	0.74	7	0.51	28	0.66	13	0.13	17	1.26	32
6	IG195	0.41	1	1.3	41	2.83	40	0.58	41	1.62	41
7	IG462	1.35	35	0.4	22	1	24	0.2	28	0.98	16
8	IG590	1.17	27	0.82	39	1.92	34	0.39	38	1.12	36
9	IG857	1.26	30	0.62	33	1.32	28	0.24	29	0.86	33
10	IG156514	1.14	26	0.26	12	0.19	4	0.03	4	1	19
11	IG156633	0.98	19	0.48	26	0.47	8	0.09	12	1.13	30
12	IG156635	0.48	3	1.67	42	5.19	42	0.92	42	1.6	42
13	IG156648	0.74	6	0.63	34	0.77	16	0.09	11	1.42	37
14	IG156656	0.88	12	0.6	31	0.89	20	0.15	22	1.23	34
15	IG156771	1.21	28	0.48	25	0.95	21	0.19	26	1.03	25
16	IG2684	1.03	22	0.37	19	0.53	10	0.09	13	1.05	23
17	IG4400	0.99	20	0.15	5	2.15	37	0.14	19	1.25	3
18	IG4401	1.25	29	0.47	24	0.72	14	0.15	21	0.86	29
19	IG4605	0.9	14	0.83	40	2.86	41	0.47	39	1.55	27
20	IG5244	0.95	18	0.46	23	0.36	6	0.07	7	1.11	31
21	IG5562	0.45	2	0.79	37	1.42	29	0.29	32	1.56	38
22	IG5588	1.08	25	0.31	13	0.78	17	0.14	20	1.06	13
23	IG69492	1.06	24	0.36	17	0.09	1	0.01	1	1.06	28
24	IG70079	0.82	10	0.77	36	1.79	31	0.28	31	1.42	35
25	IG71366	1.05	23	0.36	16	2.08	36	0.29	33	1.16	6
26	IG75929	1.32	33	0.22	9	0.15	3	0.03	3	0.88	15
27	IG75932	0.85	11	0.57	30	1.97	35	0.36	36	1.25	21
28	IG76251	0.8	9	0.38	21	0.48	9	0.07	8	1.3	24
29	IG114663	1.44	37	0.22	8	0.25	5	0.05	5	0.84	11
30	IG114670	0.93	16	0.18	6	2.17	38	0.07	9	1.25	1
31	IG114703	0.91	15	0.81	38	1.27	27	0.26	30	1.1	39
32	IG115370	1.27	31	0.35	15	0.39	7	0.08	10	0.96	22
33	IG117684	1.31	32	0.38	20	1.92	33	0.31	34	1.07	8
34	ILL8009	1.35	34	0.56	29	1.88	32	0.37	37	1.08	18
35	IG138106	1.55	39	0.35	14	0.58	12	0.12	15	0.79	20
36	ILX87075	1.66	41	0.1	1	0.99	23	0.14	18	0.76	4
37	L24	0.9	13	0.61	32	2.5	39	0.51	40	1.25	14
38	IG70056	0.58	4	0.73	35	0.74	15	0.05	6	1.44	40
39	2009S 96568-1	0.58	5	0.37	18	0.8	18	0.16	23	1.64	17

40	IG156801	1.54	38	0.23	10	1.04	25	0.16	24	0.81	9
41	010S 96130-1	0.93	17	0.51	27	0.99	22	0.18	25	1.29	26
42	010S 96155-2	1.03	21	0.26	11	0.84	19	0.13	16	1.14	10

Highlighted in bold are the 10 most stable accessions, R_{CS}: Ranking of accessions based on Cultivar Superiority, R_{SS}: Ranking of accessions based on Static Stability, R_{WE}: Ranking of accessions based on Wricke's Ecovalence, R_{FW}: Ranking of accessions based on Finlay and Wilkinson and R_{SH}: Ranking of accessions based on Shukla.

To statistically compare the five stability parameters, Spearman’s coefficient of rank correlation was calculated; it ranged from -0.6 and 0.87, indicating a wide range of variation in the performance of the accessions across the parameters. There was highly significant but negative correlation between cultivar superiority, Finlay and Wilkinson (-0.57), and between cultivar superiority and static superiority (-0.60). Conversely, highly significant and positive correlations existed between Shukla and Wricke’s Eco-valence (0.87), which identified eight stable accessions: IG114663, IG115370, IG156514, IG257, IG5244, IG69492, IG75929, and IG76251. Furthermore, a positive correlation existed between Static Stability and Finlay and Wilkinson identifying three stable accessions: IG114663, IG257 and IG75929. Four stable accessions were identified by Static stability and Shukla: IG114663, IG114670, IG257 and IG75929, with Static stability and Wricke's Ecovalence identifying three stable accessions: IG257, IG75929 and IG114663. were the most stable genotypes as they ranked among the top ten most stable genotypes based on a variety of parameters. Nevertheless, the rankings of the identified stable genotypes vary from one parameter to another despite their positive correlation (Table 5).

Table 5. Spearman's coefficients of rank correlation for five stability parameters analyzed for grain yield of lentil accessions tested in different environments.

	Cultivar Superiority	Finlay and Wilkinson	Shukla	Static Stability
Finlay and Wilkinson	-0.57***	-		
Shukla	-0.17	0.15	-	
Static Stability	-0.60***	0.87***	0.52***	-
Wricke's Ecovalence	-0.24	-0.01	0.87***	0.39*

*** $p<0.001$, * $p<0.05$

3.4. GGE-biplot

A GGE biplot was conducted for seed yield per plant (SY) traits to assess the reproducibility of the tested lentil accessions and determine which-won-where pattern. The biplot accounted for 60.79% of the variation (Figure 2). The environments E0 and E1 were omitted from the

GGE biplot analysis due to low heritability and consequently low variability, which may not be due to genetic variation but rather environmental conditions.

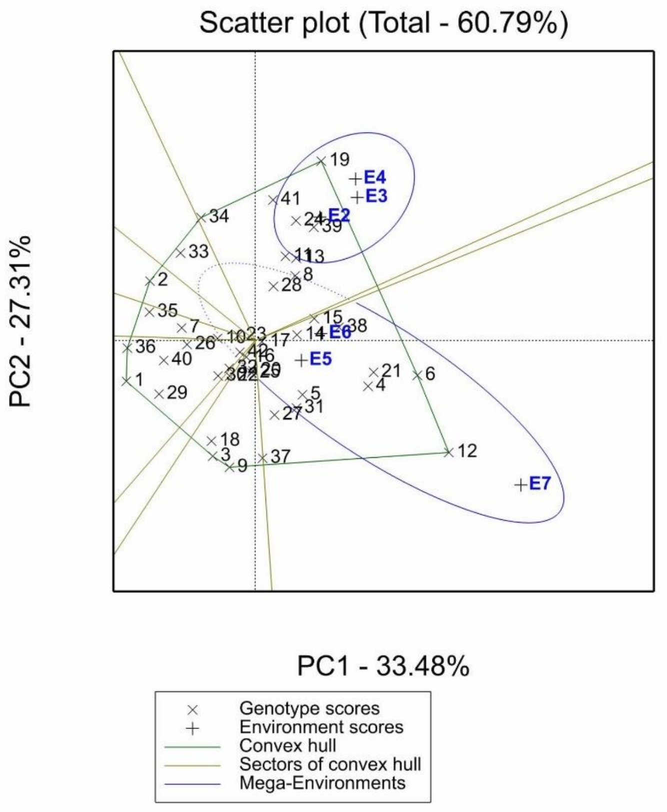


Figure 2. GGE biplot of tested accessions in validation trials for yield data (SY) explained 60.40% of total variability. E2: Marchouch-2016/17-imazethapyr at 75 g *a.i. ha*$^{-1}$, E3: Marchouch-2016/17-metribuzin at 210 g *a.i. ha*$^{-1}$, E4: Marchouch-2016/17-no herbicide treatment, E5: Terbol-2018/19-imazethapyr at 75 g *a.i. ha*$^{-1}$, E6: Terbol-2018/19-metribuzin at 210 g *a.i. ha*$^{-1}$ and E7: Terbol-2018/19-no herbicide treatment. Accessions numbered 1 to 42 were listed in Table 5.

GGE biplot revealed that the environments E2, E3 and E4 were highly correlated as were the environments E5, E6 and E7. However, E4 and E7 have the weakest correlation and the greatest angle between their vectors.

As the GGE biplot provides an indication of the discriminating ability of each test environment based on the vector length, the E7 environment was the most discriminating for the tested genotypes, whereas the E5 environment was the least discriminating.

The GGE biplot also displays a polygon view depicting the distribution of genotypes with some genotypes located on the polygon's vertex and located within it. The genotypes located on the polygon's vertex are the farthest ones from the biplot's origin compared to those located on polygon's similar sectors. Therefore, they are considered as the most responsive ones. The genotypes located on the vertex were IG1455, IG2445, IG257, IG195, IG857, IG156635, IG4605, ILL8009 and ILX87075 (1, 2, 3, 6, 9, 12, 19, 34 and 36). The genotypes IG1455, IG2445, IG257, IG857, ILL8009 and ILX87075 (1, 2, 3, 9, 34 and 36) were not considered winning genotypes in any of the test environments because no environments were located within the sectors of the previously mentioned vertex genotypes.

The GGE biplot also identifies the mega-environments within each; multiple environments as well as their winning genotypes reside. The GGE biplot of the SY was subdivided into nine sectors and two mega-environments (ME) located in two different sectors. Mega environment 1 (ME1) consisted of Marchouch-2016/17-imazethapyr at 75 (g *a.i.* ha^{-1}) (E2), Marchouch-2016/17-metribuzin at 210 (g *a.i.* ha^{-1}) (E3), and Marchouch-2016/17-no herbicide treatment (E4). Mega-environment 2 (ME2) consisted of Terbol-2018/19-imazethapyr at 75 (g *a.i.* ha^{-1}) (E5), Terbol-2018/19-metribuzin at 210 (g *a.i.* ha^{-1}) (E6) and Terbol-2018/19-no herbicide treatment (E7).

The GGE biplot analysis of the SY revealed that genotype IG4605 (19) is the winning genotype in the ME1 having the highest seed yield per plant, while genotypes IG195 (6) and IG156635 (12) had the highest seed yield per plant in the ME2.

3.5. Yield components ranking and stability of genotypes.

The mean environment coordination method (MEC) of this study showed that 18 genotypes were located on the right side of the mean environment ordinate, indicating that their seed yield per plant was greater than the average, whereas 24 genotypes seed yields' were less than the average. The highest yielding genotypes were IG195, IG156635 and IG4605 (6, 12 and 19), while the lowest yielding genotypes were IG1455, IG114663 and ILX87075 (1, 29 and 36). Based on the parallel projections shown in figure 3, IG156771 (15) was the most stable, had the nearest projection to the mean environment axis, whereas IG156635 (12) had the farthest projection to the mean environment axis. Accessions IG590 (8), IG156656 (14), IG156771 (15), IG4400 (17), IG76251 (28), IG70056 (38) and 2009S 96568-1 (39) had yields that were higher than or comparable to the average environment and were deemed to be relatively stable.

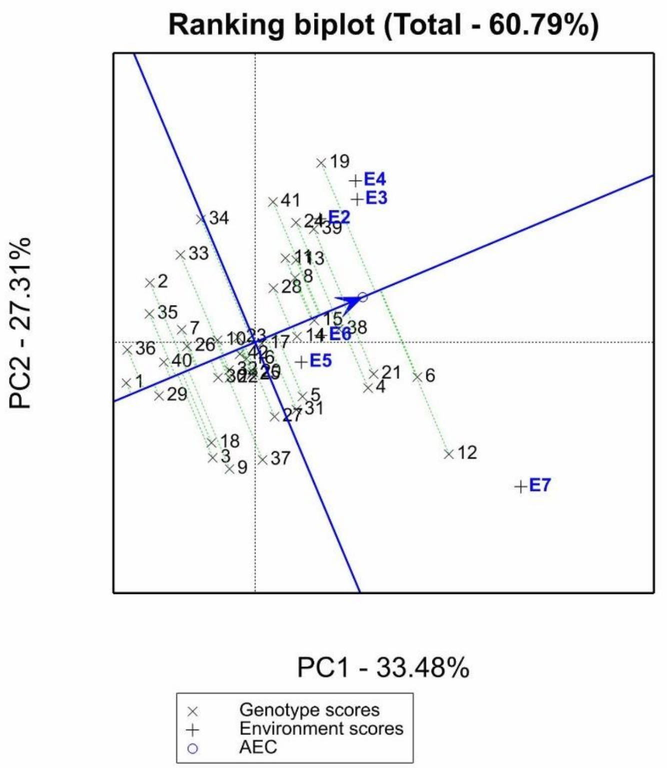


Figure 3. Average environment coordination (AEC) views of the GGE biplot show the mean yield performance and genotype stability. PC stands for principal component.

4. Discussion

Weeds are a major concern for developed and modernized farming systems that employ a small number of workers. Commercial lentil crop expansion requires machine harvestable varieties with appropriate weed management practices. Furthermore, cultivar development necessitates consistency across environments as several studies have shown that these could have multivariate responses to different environments (Dehghani *et al.* 2008; Turk and Kendal 2017). Therefore, to integrate lentil into the modernized cereals-based system, it is necessary to develop lentil cultivars that are tolerant to post-emergence herbicide and adaptable to a wide range of environments. In multi-environment trials, the performance and stability of breeding lines can be evaluated in order to identify the ideal environments for lentil screening, characterize mega environments and detect accessions with specific and broad adaptation (Gauch and Hugh 2006; Karimizadeh *et al.* 2013).

4.1. Phenological Traits

Herbicide treatment with imazethapyr and metribuzin delayed flowering and maturity in lentil, which is consistent with previous research in lentils (Balech *et al.* 2022), chickpea (Gaur *et al.* 2013b; Abou-Khater *et al.* 2021b) and faba bean (Abou-Khater *et al.* 2021b). This delay in maturity was explained by Gaur et al. (2013b) as a slowdown in crop growth rate occurred after herbicide treatment due to starvation and blockage in acetolactate synthase catalyzed reactions (Royuela *et al.* 2000). Furthermore, herbicide tolerant faba bean (Abou-Khater *et al.* 2021b) and lentil (Balech *et al.* 2022) accessions were affected by the herbicide treatment but subsequent plant growth led to recovery, resulting in further delay of flowering and maturity time.

The flowering and maturity time of lentil accessions were longer at Terbol than at Marchouch. This finding is explained by the fact that the climate at Terbol is cooler and has more precipitation than the climate at Marchouch, as reported in faba bean by (Abou-Khater *et al.* 2022b). Furthermore, heat and drought stress have been shown to shorten crop cycle duration in lentil (Choukri *et al.* 2020), chickpea (Rani *et al.* 2020) and faba bean (Maalouf *et al.* 2015). The delayed flowering and maturity observed in both treatments at Marchouch 2015/16 was expected due to an exceptional season with a lower than usual maximum temperature.

4.2. Yield Attributes

Seed yield was lower in environments treated with imazethapyr or metribuzin, than in environments not treated with herbicides. Similar findings have been previously made in lentil (Sharma *et al.* 2018; Balech *et al.* 2022), faba bean (Abou-Khater *et al.* 2021b), and chickpea (Taran *et al.* 2010). Furthermore, biological yield per plant (BY) at Marchouch in 2015/16 was lower than at Terbol in 2018/19, which was followed by Marchouch in 2016/17 due to low precipitation in January at Marchouch in 2015/16 and well-distributed precipitation from December to February at Marchouch in 2016/17 during the vegetation growth phase. For SY, the highest value was obtained at Terbol in 2018/19 no herbicide treatment (E7), which was expected given that this environment experienced high precipitation and low temperatures and no herbicide treatment during the crop season.

The heritability estimate from multi-environment trial analysis is more accurate than the estimates from a single environment. Heritability estimates for phenological traits (DF and DM) were higher than growth and yield attributes (PH, BY and SY). Lower heritability estimates for BY and SY indicated that these traits were highly influenced by environmental factors and controlled a large number of genes with a small effect when compared to phenological traits. These findings are consistent with previous research on faba bean (Abou-Khater *et al.* 2022b), chickpea (Mohammed *et al.* 2019) and lentil (Bicer and Sakar 2006).

4.3. Stability parameters

Stability parameters are used to assess genotype performance in terms of yield and stability in a variety of environments (Bicer and Sakar 2006). In the current study, five stability parameters were used to rank the genotypes in terms of stability. Previous research in lentils and other crops compared stability parameters to advise the breeders on the best method to use for selection. Our findings revealed inconsistencies in genotype ranking, as previously reported in faba bean (Abou-Khater *et al.* 2022b), lentil (Bicer and Sakar 2006; Mohebodini *et al.* 2006), chickpea (Yadav *et al.* 2010) and sorghum (Adugna 2007). Nevertheless, the analysis of the Spearman’s coefficient revealed that there were some correlations between these stability parameters. Dehghani et al. (2008) made similar observations about the similarity between Wricke’s Ecovalence and Shukla parameters but they disapproved about the similarity between Finlay and Wilkinson and Static Stability. Furthermore, several studies have confirmed the ability of cultivar superiority index to select genotypes with high and stable yield (Makanda *et al.* 2010; Shiringani and Shimelis 2011). The most stable genotypes were identified using static stability, Wricke's eco-valence, Shukla and Finlay, and Wilkinson parameters across all test environments (Fasahat 2015; Ramazani *et al.* 2016). However, our study found that the cultivar superiority parameter was not related to any of the other parameters studied and was also negatively correlated with Static Stability and Finlay and Wilkinson’s parameters. Mustapha and Bakari (2014) and Abou-Khater et al. (2022b) obtained similar observations. In our study, three accessions IG257, IG75929 and IG114663, were identified as the most stable genotypes by static stability, Wricke’s eco-valence, Shukla and Finlay’s and Wilkinson’s parameters, as well as being moderately to highly tolerant to imazethapyr and metribuzin. The cultivar superiority parameter, however, ranked these genotypes among the least stable. As a result, selecting stable and high yielding genotypes would necessitate the use of more than one parameter (Westcott 1986).

4.4. GGE Biplot, Ranking and comparison with Stability Parameters

Breeding lines with a narrow genetic base are typically less stable than those with a broad genetic base (Roy *et al.* 2013). Stable genotypes are well adapted to a wide range of environments, whereas unstable genotypes have limited adaptability. A genotype is considered stable if it contributes little to GE interaction (Fasahat 2015). Environmental conditions have been shown to influence herbicide response in faba bean (Abou-Khater *et al.* 2022b), soybean (Stewart *et al.* 2010) and corn (Stewart *et al.* 2012) .

In this study, the GGE biplot was used to graphically display genotype stability and GE interaction under various test environments. The GGE biplot depicted more than 60% of the total variability. Thus, the biplot can safely be interpreted as effective graphical representation of MET data variability and the correlations between two environments are reliable (Rakshit *et al.* 2012). GGE biplot was performed on six environments (E2, E3, E4, E5, E6 and E7) in this study; E0 and E1 with low heritability were excluded because they accounted for less than 60% variability when included as described in other studies.

Marchouch (E2, E3 and E4) and Terbol (E5, E6 and E7) environments were correlated with an angle less than 90º in this study (Yan *et al.* 2000). Terbol-2018/19-no herbicide treatment (E7) was the most discriminating environment, and the least discriminating environments were those treated with imazethapyr and metribuzin (E2, E5 and E6). As a result, the genotypes tested in this study were heavily influenced by the location and herbicide treatment. This is because of the warm and dry weather at Marchouch, where a combination of herbicide treatment and environmental conditions affects the accessions. Therefore, the best test environment for screening lentil accessions for stability of agronomic performance should be in an environment that is less likely to experience stress periods such as Terbol.

A mega-environment is defined as a group of environments that share the best set of genotypes in terms of performance repeatability and consistency (Yan *et al.* 2007). The environments within the same mega-environments (ME1 and ME2) in our study were consistent with the climatic conditions. Faba bean (Abou-Khater *et al.* 2022b) and sorghum (Rakshit *et al.* 2012) yielded comparable results. This confirms that the GE interaction was influenced more by the climatic conditions of the location than by the herbicide treatment.

According to Yan et al. (2007), the most responsive genotypes may have the highest or lowest seed yield per plant (SY), but the ideal winning genotype has a high mean yield and high stability (Yan and Rajcan 2002). The GGE biplot of genotypes in this study revealed that IG195, IG156635 and IG4605 were the winning accessions with the highest adaptability in ME1 and ME2. Several studies have used the GGE biplot method to identify ideal genotypes in specific environments including maize (Fan *et al.* 2007), barley (Dehghani *et al.* 2006), wheat (Kaya *et al.* 2006), chickpea (Erdemci 2018), pea (Rubiales *et al.* 2021a) and lentil (Rubiales *et al.* 2021b). Our findings were consistent with the ranking of cultivar superiority, which identified the same three winning genotypes and ranked them among the top 15. This supported the findings of Lin and Binns (1988); Makanda et al. (2010) and Shiringani and

Shimelis (2011) regarding the ability of cultivar superiority to select the genotypes with combined ability of high stability and yield.

However, IG195, IG4605 and IG156635, the most adapted accessions in ME1 and ME2, were not considered stable by the biplot ranking. Yan and Rajcan (2002) reported that an ideal genotype has a high mean yield and a high stability across environments. A genotype may be highly stable across the test environments but low yielding or vice versa. In this study, the ranking biplot identified IG70056 (38) as having a high yield as well as being highly stable. Other stability parameters, such as Cultivar superiority and the Shukla parameter, ranked IG70056 (38) among the top ten stable lines including.

5. Conclusions

To increase the accuracy of selection of superior genotypes, yield and stability of performance across environments should be taken into consideration rather than depending only on the average of performance. This study was based on multi-environment trials in which, five stability parameters showed inconsistency in ranking the genotypes despite the existence of positive correlations between some of them. Some accessions with higher-than-average yields were classified as unstable, while others with low yields were classified as highly stable. Static Stability, Finlay and Wilkinson, Wricke's Ecovalence and Shukla parameters identified low yielding genotypes as stable, whereas GGE biplot and cultivar superiority index ranked the genotypes similarly in terms of yield. GGE biplot identified IG70056 (38) as a superior line with high and stable yield across years and locations due to its tolerance to imazethapyr and metribuzin. IG4605 (19), IG195 (6) and IG156635 (12) were discovered to be specifically adapted to one mega environment. Furthermore, to avoid the confounding effect, this study recommends conducting herbicide screening trials in environments that do not experience drought periods.

To summarize, in order to develop superior herbicide tolerant genotypes that are adapted to various mega environments, it is necessary to cross tolerant genotypes having stable performance with genotypes adapted to specific environments or having traits of economic interest.

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Chapter IV

Identification of novel genes associated with herbicide tolerance in Lentil (*Lens culinaris* ssp. *culinaris* Medik.

1. Introduction

Lentil (*Lens culinaris* ssp. *culinaris* Medik.) is an important legume widely grown in many countries. Being versatile in cooking and a good source of protein as well as various micronutrients, lentil is an essential element for human health and a major component for cereal and rice based alimentary diets (Johnson *et al.* 2015). In addition, its straw has a nutritional value as animal feed (Landero *et al.* 2012). Lentil improves soil fertility by its capacity to fix nitrogen and increases soil aeration through its shallow root system (Cokkizgin and Shtaya 2013).

During the past two decades, the cultivation of lentil has been expanded to new areas by 28% leading to 42% increase in production as well as 18% increase in yield (FAOSTAT 2023). The yield improvement and cultivation expansion to new regions are the result of the development of appropriate varieties for various market segments and application of good agronomic practices. Despite these achievements, various biotic and abiotic stresses are still affecting its productivity in farmers' fields such as heat, drought, diseases, and poor weed management. Parasitic and annual broad-leaved weeds cause significant yield losses up to 95%, especially when mismanaged (Elkoca *et al.* 2005b; Rubiales and Fernández Aparicio 2012). Herbicide tolerance is the most effective technique to control weeds in lentils as other techniques are expensive and time consuming. Sources of tolerance to pre-emergence herbicides (metribuzin and imazethapyr) were identified in lentil (Sharma *et al.* 2017b, 2018; Balech *et al.* 2022) and other crops such as faba bean (Abou-Khater *et al.* 2021b), chickpea (Gaur *et al.* 2013b), and soybean (Stewart *et al.* 2010). Currently, the major efforts for developing herbicide tolerant lentil breeding lines were made through field selection with limited progress due to the low selection accuracy of visual assessment. Improving selection accuracy can be achieved by the utilization of modern breeding methods such as markers assisted selection (MAS) and genomic selection (Kumar *et al.* 2015).

Lentil is a diploid (2n=14) and self-pollinating crop with a large genome size of 4 gigabases (Gb) (Arumuganathan and Earle 1991); its genome is larger than many previously sequenced crops like soybean, chickpea, maize, and rice. However, lentil genome sequencing is possible today due to advances in sequencing technologies and bioinformatics tools (Bett 2016). In fact, several linkage maps have been constructed and used for the identification of many genes and quantitative trait loci (QTL) controlling a range of biotic and abiotic traits (Verma *et al.* 2015; Sudheesh *et al.* 2016; Khazaei *et al.* 2017; Singh *et al.* 2019). However, these markers have been proved of limited value due to their narrow association with biparental genetic backgrounds. Genome wide association mapping (GWAS) is an alternative approach that utilize genome-wide single nucleotide polymorphism (SNP) markers for the identification of marker trait associations in diverse germplasm panels (Kumar *et al.* 2015). Studies have showed that the optimal implementation of single trait GWAS is under controlled conditions involving one environment and allowing to differentiate between genetic and environmental effect. Moreover, the single trait GWAS doesn't dissect the presence of correlated traits or pleiotropic effect in contrary to the meta-GWAS approach (Merrick *et al.* 2022). However, the results of multiple single-trait GWAS statistics can be combined using meta-GWAS approach (Evangelou and Ioannidis 2013) to increase the population size and consequently improve the power of the GWAS analysis (Bolormaa *et al.* 2014). Most meta-GWAS methods required only the SNP effects, calculated using single-trait GWAS for different variants, and their standard errors to calculate a global p-value that is equivalent to the one calculated when combining the actual phenotypic and genotypic data for all variants (Joukhadar and Daetwyler 2022).

The purpose of this study is to deploy meta-GWAS analysis to identify SNPs markers associated with herbicide damage as well as different agronomic traits of lentil with and without herbicide treatments using multilocation/season phenotypic data.

2. Materials and methods

2.1. Materials and experiments

A set of 292 lentil accessions including 175 landraces collected from 49 countries, and 117 breeding lines developed at ICARDA were evaluated to their response to imazethapyr and metribuzin treatments, separately at different doses.

Four field experiments were conducted at Marchouch, Morocco (33.56°N, 6.69°W) during 2013/14 and 2014/15 and at Terbol, Lebanon (33.81°N, 35.98°E) during 2014/15 (Balech *et al.* 2022) and 2019/20, in alpha lattice design with two replicates and a plot size of 1 row of 1 m length spaced at 0.3 m distance. The details of each experiment and the applied herbicide treatments at the pre-flowering stage are presented in Table 1.

Table 1. Environmental conditions of different location-season-treatment combinations of lentil screening.

Location-Season	Treatment	Soil Type	Rainfall (mm)	Air Temperature (°C)		
				Average (AVG)	AVG Min	AVG Max
Marchouch-2013/14	Imazethapyr 37.5 (g a.i.ha^{-1})	Vertisols and silty clay	248	16.5	8.7	20.1
	Imazethapyr 75 (g a.i.ha^{-1})					
	Imazethapyr 112.5 (g a.i.ha^{-1})					
Marchouch-2014/15	Imazethapyr 75 (g a.i.ha^{-1})	Vertisols and silty clay	291	14.1	8.5	19.7
	Imazethapyr 150 (g a.i.ha^{-1})					
	Metribuzin 210 (g a.i.ha^{-1})					
	Metribuzin 420 (g a.i.ha^{-1})					
	No herbicide treatment					
Terbol-2014/15	Imazethapyr 112.5 (g a.i.ha^{-1})	Clay loam	421	9.8	2.8	16.9
	Metribuzin 315 (g a.i.ha^{-1})					
Terbol-2019/20	Imazethapyr 112.5 (g a.i.ha^{-1})	Clay loam	671	10.2	3.5	17.1
	Metribuzin 315 (g a.i.ha^{-1})					
	No herbicide treatment					

2.2. Phenotypic Data for Herbicide Tolerance

Based on the Lentil ontology (Kumar and Rajendran 2016) the following phenotypic data were recorded:

Herbicide damage score (HDS) was recorded using the scale described in Balech et al. (2022)on a scale of 1 to 5, at two weeks (HDS1) and then at five weeks (HDS2) after the herbicide application at Terbol in 2014/15 but at Marchouch in 2013/14, only HDS2 was recorded. This scale was proposed by to assess the ability of accessions to recover from herbicide treatments.

Crop phenology traits of number of days to 50% flowering (DF) and days to 95% of maturity (DM) from sowing day were recorded on a plot basis at Terbol in 2014-15 and 2019/20.

Agronomical and yield traits of plant height (PH) (cm), biological yield/plant (BY) (g) and seed yield/plant (g) data were recorded on three randomly selected plants per plot and the average was calculated from trials at Marchouch 2014/15, Terbol 2014/15 and 2019/20. In addition, the number of pods/plant (NPP) was also recorded and calculated as PH, BY and SY at Terbol 2019/20.

The reduction indices: The reduction index (RI_{trait}) was estimated to measure the performance of selected tolerant accessions, as follows (Sharma *et al.* 2018):

$$RI_{trait} = 100 - \frac{(100 \times \overline{\mathrm{T}})}{\overline{\mathrm{C}}}$$

Where ($\overline{\mathrm{T}}$) is the trait value of evaluated accession under herbicide treatments and $\overline{\mathrm{C}}$ is the value of the same accession under controlled conditions without any herbicide treatments. This reduction index was calculated for DF, DM, PH, NPP, BY and SY at Terbol in 2019/20. At Marchouch in 2014/15, only the reduction indices for PH, BY and SY were calculated.

2.3. DNA extraction and genotyping by sequencing analysis

DNA was extracted from young leaves of seedlings aged between 4 to 6 weeks, prior to the application of salt treatment, using the CTAB method, as outlined by Rogers and Bendich (1985). A total of 50 µl of 100 ng/µl DNA from each sample was sent to Agriculture Victoria, Melbourne, where Multispecies Pulse SNP chip was used for genotyping and 10,271 SNP were detected. To ensure the quality of the markers, we filtered them by call rates greater than 80%, minor allele frequency (MAF) of $\geq 5\%$, and heterozygosity of $\leq 15\%$. Only those markers that met these criteria were selected for genome-wide association analysis.

2.4. Phenotypic data analysis

The spatial statistical row-column model was used to detect differences among genotypes (G) under different herbicide treatments (T), location (L) and their interactions (G x T), (G x L) and (G x T x L) for phenological and agronomic traits using Genstat V. 19 (Goedhart and Thissen 2018). The significance of variation among accessions and herbicide treatments was tested using p values. The best linear unbiased prediction values (BLUP) of genotypes and treatment and interactions between genotypes and treatments were also estimated by Genstat V. 19.

2.5. Genetic Diversity study

The phylogenetic data analysis was carried out using the programming language R, using the clust agglomeration method of "complete". The similarity data matrix obtained from the SNP genotyping data was then used to construct the phylogenetic tree. To visualize the tree, we used the online tool iTOL (Interactive Tree Of Life), which allowed us to color-code the samples based on their country of origin and provided a user-friendly interface for exploring and analysing the data (Figure 1).

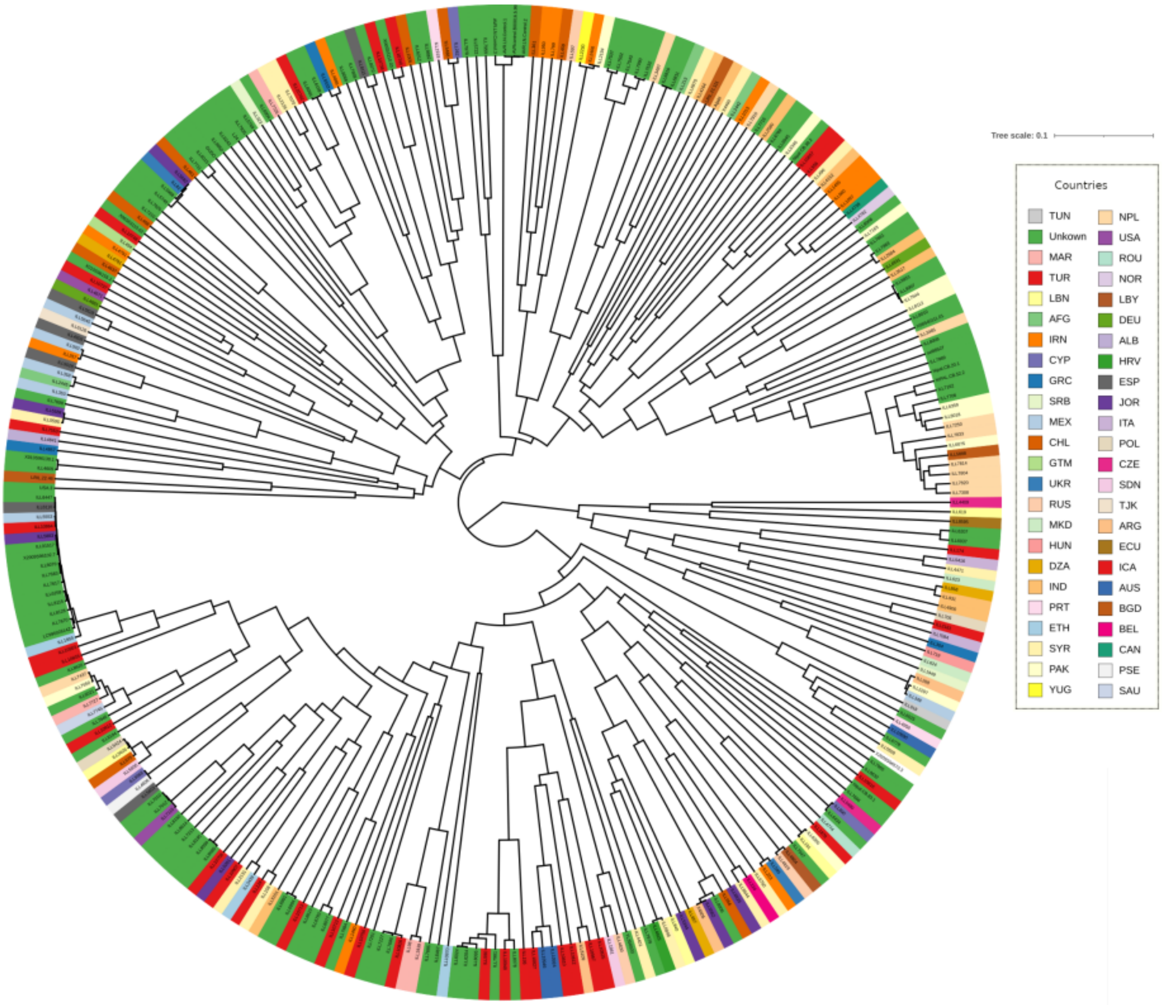

Figure 1. Phylogenetic tree of the studied lentil accessions using SNP genotyping data. The samples are color-coded based on their country of origin.

Single-trait GWAS

The single variate mixed linear model implemented in the software GEMMA (Zhou and Stephens 2012, 2014) was used to analyze the association between each measured phenotype in each environment with the (Zhou and Stephens 2012, 2014)78; https://doi.org/10SNP data. The model used the following equation:

$$y = \mu + X\beta + I\alpha + e$$

Where y is a vector of the phenotypes, μ is the intercept, X is the incidence matrix assigning individuals to genotypes, β is the SNP substitution effect, I is the identity matrix, α is a vector of random effects, and e is a vector for the residuals.

Meta-GWAS

Meta-GWAS analysis was performed following the method described in Bolormaa et al. (2014). Briefly, the following equation was used to calculate a chi-squared statistic (X^2) assuming n (number of environments per trait) degrees of freedom:

$$X_i^2 = t_i' V^{-1} t_i$$

Where t_i represents the signed t-values for the SNP (i) in all environments, and $V^{-1}$is the inverse of the correlation of the t-values among all environments. The following equation was used to calculate t_i:

$$t_i = \frac{b_i}{se(b_i)}$$

Where b_i is the SNP effect calculated in the single-trait GWAS analysis for each environment and $se(b_i)$ is its standard error. Bonferroni correlation was used to declare significance. However, all associations with p<0.0001 were reported in the supplementary materials as suggestive associations.

3. Results

3.1. Phenotypic Results

3.1.1. Herbicide damage Score

HDS1 and HDS2 scores ranged from 1 to 5 for imazethapyr and metribuzin at different dosages showing significant variation for herbicide tolerance among the lentil accessions. In Marchouch 2014/15 and after two weeks of applying imazethapyr at 75 g a.i.ha^{-1}, 1% of the total accessions scored 2 with slight damage on leaves with marginal yellowness, 77% scored 3 with moderate damage with leaf necrosis, 18% scored 4 with severely damaged with 25% to 75 % mortality, and 5% scored 5 with total mortality. HDS2 score taken after five weeks of herbicide treatments showed recovery of the injuries in the accessions; 3% of total tested accessions with marginal leaf yellowness recorded 2 score, 88% with moderate damage scored 3, 9% accessions with severe damage scored 4, and none accession scored 5. When applying imazethapyr at 150 g a.i.ha^{-1}, HDS1 scored 3 (21% accessions), 4 (57% accessions) and 5 (22% accessions); confirming that the damages were more severe at higher dosage. After five weeks of the treatment, HDS2 scored 3, 4 and 5 in 2%, 51% and 47%, of the accessions, respectively showing that no recovery occurred. In Terbol 2014/15 and after two weeks of applying imazethapyr at 112.5 g a.i.ha^{-1}, HDS1 scored 2 (10% accessions), 3 (55% accessions) and 4

(35% accessions). Whereas HDS2 scored 2 (6% accessions), 3 (40% accessions), 4 (48% accessions) and 5(6% accessions) showing that the toxicity symptoms were aggravated. The observations made during Terbol 2019/20 at the same dosage of imazethapyr (112.5 g a.i.ha^{-1}) showed that the toxicity symptoms were less than the symptoms that occurred during Terbol 2014/15 and HDS2 ranged between 1 and 4 showing that the accessions recovered (Figure 2).

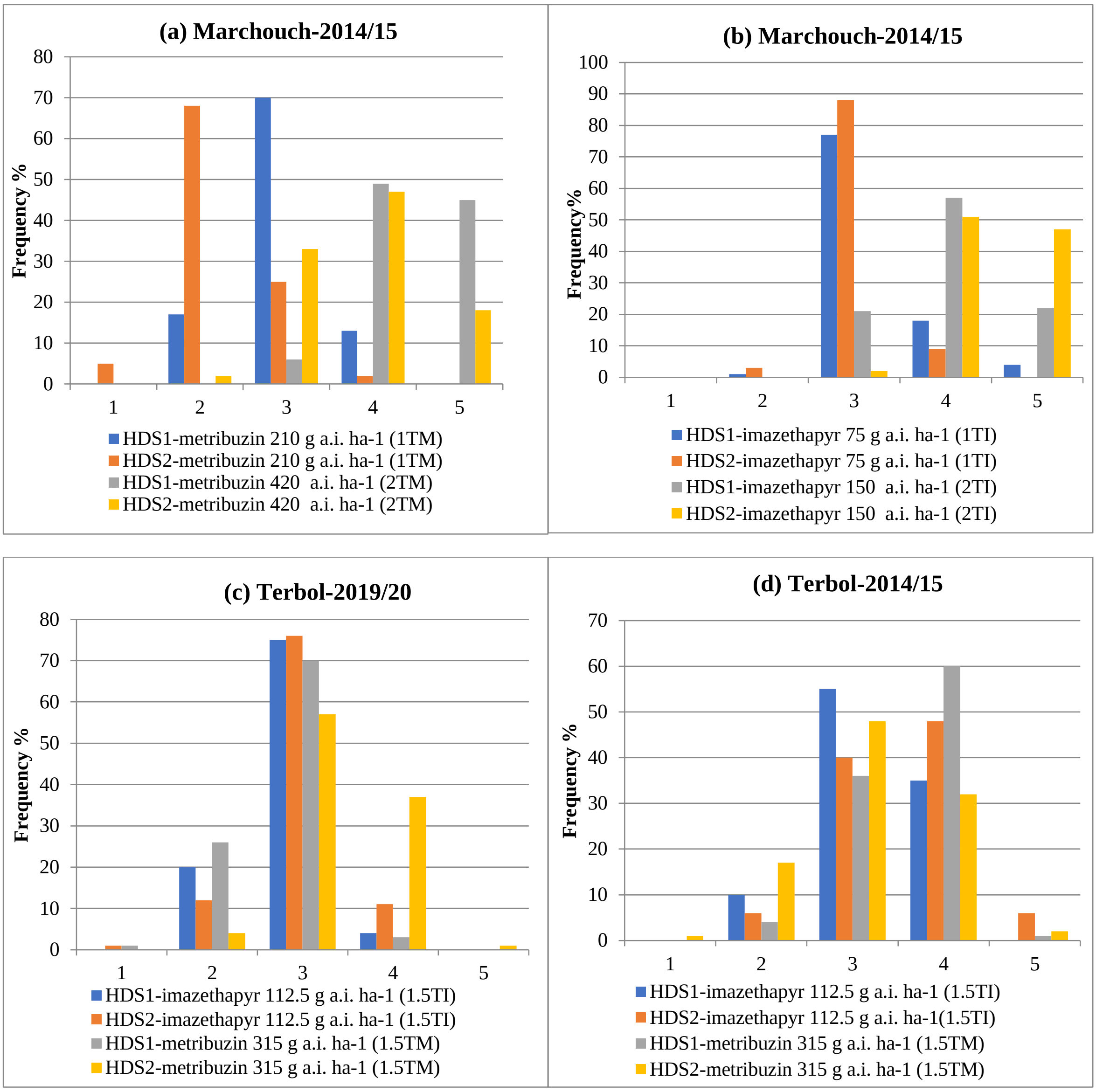

Figure 2. Distribution of lentil genotypes for herbicide damage scores (HDS1 and HDS2) under different dosages of imazethapyr and metribuzin during different locations and cropping seasons.

For metribuzin at 210 g a.i.ha^{-1} treatment, HDS1 showed wide variation with 17% of the total accessions scoring 2 with minimum damage (marginal leaf burning), 70% scoring 3 with moderate damage (leaf necrosis and lower vegetative growth), 13% scoring 4 with high damage (severe leaf burning). HDS2 score showed recovery from the herbicide damage with the formation of new leaves. HDS2 score showed that 5% of total accessions scored 1 with no visible damage, 68% scored 2 with slight damage, 25% scored 3 with moderate damage and 2% scored 4 with a mortality rate between 25 and 75%. Similar to imazethapyr, when doubling the dosage of metribuzin (420 g a.i.ha^{-1}), HDS1 ranged between 3 and 5 showing aggravation of toxicity symptoms. Five weeks after the treatment, HDS2 ranged between 2 and 5 showing recovery of the toxicity symptoms. During Terbol 2014/15, when metribuzin (315 g a.i.ha^{-1}) was applied, HDS1 scored 2 (10%), 3 (55%) and 4 (35%) while, HDS2 scored 2 (6%), 3 (40%), 4 (48%) and 5 (6%) showing that the toxicity symptoms were aggravated. The observations made during Terbol 2019/20 at the same dosage of metribuzin (315 g a.i.ha^{-1}) showed that the toxicity symptoms were less than the ones occurred during Terbol 2014/15 while HDS2 ranged between 2 and 5 showing that the toxicity symptoms aggravated (Figure 2).

3.1.2. Crop phenology, yield and yield components

The combined variance analysis revealed $p < 0.001$ among the accessions (G) indicating that the tested germplasm was significantly diverse. Moreover, significant differences also existed among treatments (T) and locations (L) for all the traits except for the number of pods per plant (NPP). The interaction between genotype x treatment (G x T) across trials and between Genotype x Location (G x L) across treatments was also significant. The Genotype × Treatment × Location (G x T x L) interaction showed that the genotypes response to the effect of herbicide treatments was not affected by the environment except for DF and DM and their reduction indexes (Tables 2 and 3).

Table 2. Combined analysis performed for detecting differences among genotypes (G), Location (L), Treatments (T) and G x T, G x L and G x T x L interactions for phenological and agronomic traits performed for the trials at Marchouch during 2014/15 and at Terbol during 2014/15 and 2019/20.

	DF	**DM**	**PH**	**BY**	**SY**	**NPP**
Genotype (G)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment (T)	<0.001	<0.001	<0.001	<0.001	<0.001	0.07
G x T	<0.001	<0.001	0.003	0.023	0.04	0.06
Location (L)	<0.001	<0.001	<0.001	<0.001	<0.001	ND
G x L	<0.001	<0.001	<0.001	<0.001	<0.001	ND
G x T x L	<0.001	<0.001	0.936	0.987	1	ND

DF: days to 50% flowering, DM: days to maturity, PH: plant height, BY: biological yield per plant, SY: seed yield per plant, NPP: number of pods per plant, ND: not defined.

Table 3. Combined analysis performed for detecting differences among genotype (G), Location (L), Treatment (T) and G x L interactions for reduction indexes (RI) of phenological and agronomic traits performed for the trials at Marchouch during 2014/15 and at Terbol during 2014/15 and 2019/20.

	RI_{DF}	RI_{DM}	RI_{PH}	RI_{BY}	RI_{SY}	RI_{NPP}
Genotype (G)	0.9	0.6	<0.001	<0.001	<0.001	<0.001
Treatment (T)	<0.001	<0.001	<0.001	<0.001	0.02	0.4
Location (L)	ND	ND	<0.001	<0.001	<0.001	ND
G x L	ND	ND	<0.001	0.1	0.2	ND

RI_{DF}: reduction index for days to 50% flowering, RI_{DM}: RI for days to maturity, RI_{PH}: RI for plant height, RI_{BY}: RI for biological yield per plant, RI_{SY}: RI for seed yield per plant, RI_{NPP}: RI for number of pods per plant, ND: not defined.

During Terbol 2019/20, plant height (PH) was significantly less in plots treated with imazethapyr at 112.5 g a.i.ha^{-1}. Similar observation was observed during Marchouch 2014/15 when treated with imazethapyr at 70 g a.i.ha^{-1} recording a reduction of 28.8% (Table 4 and 5). The reduction in plant height was severe when imazethapyr was applied at higher dosage (140 g a.i.ha^{-1}). When metribuzin applied at 210 g a.i.ha^{-1} and 315 g a.i.ha^{-1} respectively at Marchouch 2014/15 and Terbol 2019/20, the plant height was not significantly reduced in comparison to the untreated plots. On the other hand, when applying Metribuzin at 420 g a.i.ha^{-1}, PH was significantly lower (by 33%) than the untreated plots (Table 4 and 5).

Table 4. Mean ± Standard error (SE) for different traits under different environments and treatments.

Treatment	DF	DM	PH	BY	SY
			Terbol-2014/15		
Imazethapyr at 112.5 g a.i.ha^{-1}	124.8 ± 8.7	168.2 ± 5.5	17.7 ± 4.6	1.7 ± 0.8	0.2 ± 0.2
Metribuzin at 315 g a.i.ha^{-1}	119.5 ± 8.7	166.6 ± 5.5	18.6 ± 4.6	1.5 ± 0.8	0.3 ± 0.2
			Terbol-2019/20		
Imazethapyr at 112.5 g a.i.ha^{-1}	162 ± 9.1	207.4 ± 2.8	27 ± 3.9	3.4 ± 1.5	0.8 ± 0.6
Metribuzin at 315 g a.i.ha^{-1}	133.1 ± 9.1	194.2 ± 2.8	29 ± 3.9	2.5 ± 1.5	0.9 ± 0.6
No herbicide treatment	131.7 ± 9.1	191.2 ± 2.8	35.5 ± 3.9	3.5 ± 1.5	1.3 ± 0.6
			Marchouch-2014/15		
Imazethapyr at 70 g a.i.ha^{-1}	ND	ND	26.3 ± 5.5	3.6 ± 2	0.3 ± 0.5
Imazethapyr at 140 g a.i.ha^{-1}	ND	ND	20.2 ± 5.5	1.8 ± 2	0.02 ± 0.5
Metribuzin at 210 g a.i.ha^{-1}	ND	ND	31.2 ± 5.5	4.4 ± 2	0.3 ± 0.5
Metribuzin at 420 g a.i.ha^{-1}	ND	ND	24.6 ± 5.5	1.9 ± 2	0.1 ± 0.5
No herbicide treatment	ND	ND	37.6 ± 5.5	3.7 ± 2	0.2 ± 0.5

SE: standard error, ND: Not defined, DF: days to 50% flowering, DM: days to maturity, PH: plant height, BY: biomass per plant, SY: seed weight per plant.

Table 5. Mean ± Standard error (SE) for reduction indexes (RI) of different traits under different environments and treatments.

Treatment	RI_{PH}	RI_{BY}	RI_{SY}	RI_{DF}	RI_{DM}	RI_{NPP}	RI_{NSP}
				Terbol-2019/20			
Imazethapyr at 107.5 g a.i.ha^{-1}	22.8 ± 13.77	-12 ± 65.9	25.1 ± 61.8	-9.6 ± 15.3	-3.2 ± 21.5	-30.6 ± 308.5	20 ± 70.6
Metribuzin at 315 g a.i.ha^{-1}	16.6 ± 13.77	15.6 ± 65.9	15.4 ± 61.8	-0.3 ± 15.3	5.6 ± 21.5	-6.4 ± 308.5	14.2 ± 70.6
				Marchouch-2014/15			
Imazethapyr at 70 g a.i.ha^{-1}	28.8 ± 18.2	6.6 ± 94.1	16.3 ± 98.6	ND	ND	ND	ND
Imazethapyr at 140 g a.i.ha^{-1}	45.1 ± 18.2	78.2 ± 94.1	101.7 ± 98.6	ND	ND	ND	ND
Metribuzin at 210 g a.i.ha^{-1}	15.9 ± 18.2	-25.7 ± 94.1	2.4 ± 98.6	ND	ND	ND	ND
Metribuzin at 420 g a.i.ha^{-1}	33 ± 18.2	58 ± 94.1	63.3 ± 98.6	ND	ND	ND	ND

SE: standard error, ND: Not defined, RI_{DF}: days to 50% flowering, RI_{DM}: days to maturity, RI_{PH}: plant height, RI_{BY}: biological yield per plant, RI_{SY}: seed yield per plant, RI_{NPP}: number of pods per plant, RI_{NSP}: number of seeds per plant.

During Terbol 2019/20, the biological yield per plant (BY) when treated with imazethapyr at 112.5 g a.i.ha^{-1} or metribuzin at 315 g a.i.ha^{-1} was not significantly lower than the untreated plots. Same observation was obtained during Marchouch 2014/15 when treated with different dosages of imazethapyr and metribuzin. However, when applying imazethapyr at 140 g a.i.ha^{-1} or metribuzin at 420 g a.i.ha^{-1}, the reduction of BY (RI_{BY}) increased to 78.2% and 58% respectively. Similar results were obtained for seed yield per plant (SY) when treated with imazethapyr or metribuzin at both locations Terbol and Marchouch. When increasing the dosage of imazethapyr at 140 g a.i.ha^{-1} or metribuzin at 420 g a.i.ha^{-1}, the reduction in SY (RI_{SY}) increased to 101.7% and 63.3% respectively.

3.2. Genotyping and population structure

A total of 10,271 SNPs markers uniformly distributed along the lentil genome were assayed using Multispecies Pulse SNP chip developed at Agriculture Victoria, Melbourne. After applying the quality control criteria, the final dataset consisted of 7,642 SNPs that were distributed along the lentil genome. The proportions of sequence variations of the SNP markers are as following: A/C (1433 SNPs), A/G (3675 SNPs), C/T (3764 SNPs), and G/T (1399 SNPs). The aim of our study was to use phylogenetic diversity to investigate the genetic relationship among this set of lentil population. Through the obtained phylogenetic tree, we identified three significant clusters that evenly accommodated the lentil accessions under investigation, but we did not observe any clustering of genotypes based on their country of origin (Figure 1).

3.3. GWAS and Annotation analyses

Among the 7,642 SNP markers that were assessed, 125 (clustered in 85 unique QTL) were found to be associated with herbicide tolerance, of which 36 (clustered in 30 unique QTL) were highly significant (Table 6) while the remaining SNPs were considered as suggestive associations (see Supplementary Table S1 online).

Table 6. Highly significant SNP-Trait associations revealed by the Meta-GWAS analysis.

QTL	SNP	Chr	-log10(p)	MAF
HDS2				
QTL013	AVR-Lc-01885.02-000213238	2	6.3	0.409
DF				
QTL012	AVR-Lc-01367.01-537371482	1	6.5	0.077
QTL026	AVR-Lc-02988.02-518425731	2	6.2	0.100
QTL039	AVR-Lc-03983.03-230295656	3	7.5	0.074
QTL039	AVR-Lc-03987.03-231578053	3	9.3	0.074
QTL034	AVR-Lc-04656.03-038315503	3	5.6	0.120
QTL044	AVR-Lc-05454.04-021398009	4	5.6	0.056
QTL051	AVR-Lc-05801.04-318079189	4	6.7	0.072
QTL061	AVR-Lc-06725.05-011593595	5	6.0	0.090
RI$_{DF}$				
QTL032	AVR-Lc-03379.02-609257610	2	7.1	0.085
QTL014	AVR-Lc-03458.02-007762915	2	10.1	0.050
QTL049	AVR-Lc-05740.04-302184757	4	5.4	0.073
QTL071	AVR-Lc-08010.06-011899372	6	8.0	0.053
RI$_{DM}$				
QTL019	AVR-Lc-02189.02-307011079	2	9.1	0.104
QTL019	AVR-Lc-02200.02-309350505	2	6.3	0.130
QTL083	AVR-Lc-10007.07-447269681	7	5.4	0.368
BY				
QTL021	AVR-Lc-02714.02-436766259	2	5.6	0.071
QTL021	AVR-Lc-02715.02-436994699	2	5.9	0.084
QTL062	AVR-Lc-06969.05-022095933	5	5.2	0.071
NPP				
QTL007	AVR-Lc-00835.01-430931278	1	6.0	0.062
QTL029	AVR-Lc-03296.02-599856144	2	5.7	0.089
RI$_{NPP}$				
QTL006	AVR-Lc-00579.01-366322027	1	5.7	0.136
QTL011	AVR-Lc-01352.01-535793448	1	6.8	0.142
QTL031	AVR-Lc-03373.02-608709301	2	6.2	0.092
QTL047	AVR-Lc-05203.04-122734802	4	8.1	0.063
QTL047	AVR-Lc-05322.04-163251061	4	5.4	0.080
QTL057	AVR-Lc-06339.04-451674618	4	5.8	0.071
QTL057	AVR-Lc-06341.04-451714023	4	5.7	0.062

QTL045	AVR-Lc-06527.04-050552783	4	7.6	0.094
QTL047	AVR-Lc-06652.04-092451825	4	5.2	0.080
QTL064	AVR-Lc-07086.05-026835995	5	6.7	0.060
QTL067	AVR-Lc-07474.05-420462935	5	5.6	0.071
QTL060	AVR-Lc-07900.05-008235645	5	5.9	0.089
QTL076	AVR-Lc-08292.06-221642340	6	5.4	0.070
QTL077	AVR-Lc-08717.06-348699147	6	5.4	0.077
NSP				
QTL025	AVR-Lc-02857.02-473502035	2	5.5	0.133

QTL: quantitative trait loci, SNP: single nucleotide polymorphism, Chr: Chromosome, MAF: minor allele frequency, HDS2: second herbicide damage score, DF: days to flowering, RI_{DF}: DFreduction index, RI_{DM}: Days to maturity reduction index, BY: Biological yield per plant, NPP: number of pods per plant, RI_{NPP}: NPP reduction index, NSP: number of seeds per plant.

Remarkably, traits like RI_{PH}, RI_{BY}, and RI_{SY} were excluded as there was no SNP associated with herbicide tolerance. Based on Bonferroni threshold (0.05/n) correction at $p > 4.6 \times 10$ −6, The SNPs with −log 10 (p value) ≥ 5.2 were considered to have significant associations; 36 SNPs markers were significantly highly associated with diverse traits of herbicide tolerance. Table 6 describes the positions and the significance of these SNP markers for the recorded traits as following: one SNP (AVR-Lc-01885.02-000213238) was associated with HDS (-log10(p) = 6.3), eight SNPs (the most significant are AVR-Lc-03987.03-231578053, AVR-Lc-03983.03-230295656 and AVR-Lc-05801.04-318079189) were associated with DF (-log10(p) = 5.6 to 9.3), four SNPs (AVR-Lc-03458.02-007762915, AVR-Lc-08010.06-011899372, AVR-Lc-03379.02-609257610 and AVR-Lc-05740.04-302184757) were associated with RI_{DF} (-log10(p) = 5.4 to 8), three SNPs (AVR-Lc-02189.02-307011079, AVR-Lc-02200.02-309350505 and AVR-Lc-10007.07-447269681) with RI_{DM} (-log10(p) = 5.4 to 9.1), three SNPs (AVR-Lc-02714.02-436766259, AVR-Lc-02715.02-436994699, and AVR-Lc-06969.05-022095933) with BY (-log10(p) = 5.2 to 5.9), two SNPs (AVR-Lc-00835.01-430931278 and AVR-Lc-03296.02-599856144) were associated with NPP (-log10(p) = 5.7 and 6.0), and fourteen SNPs (the most significant SNPs are AVR-Lc-01352.01-535793448, AVR-Lc-06527.04-050552783, and AVR-Lc-05203.04-122734802) were associated with RI_{NPP} (-log10(p) = 5.2 to 8.1). The significance of associations and the location on the chromosomes of these SNP are also presented in Manhattan plot and QQ plot (Figure 3).

Manhattan plot showed that SNP markers were dispersed randomly on the chromosomes from 1 to 7.

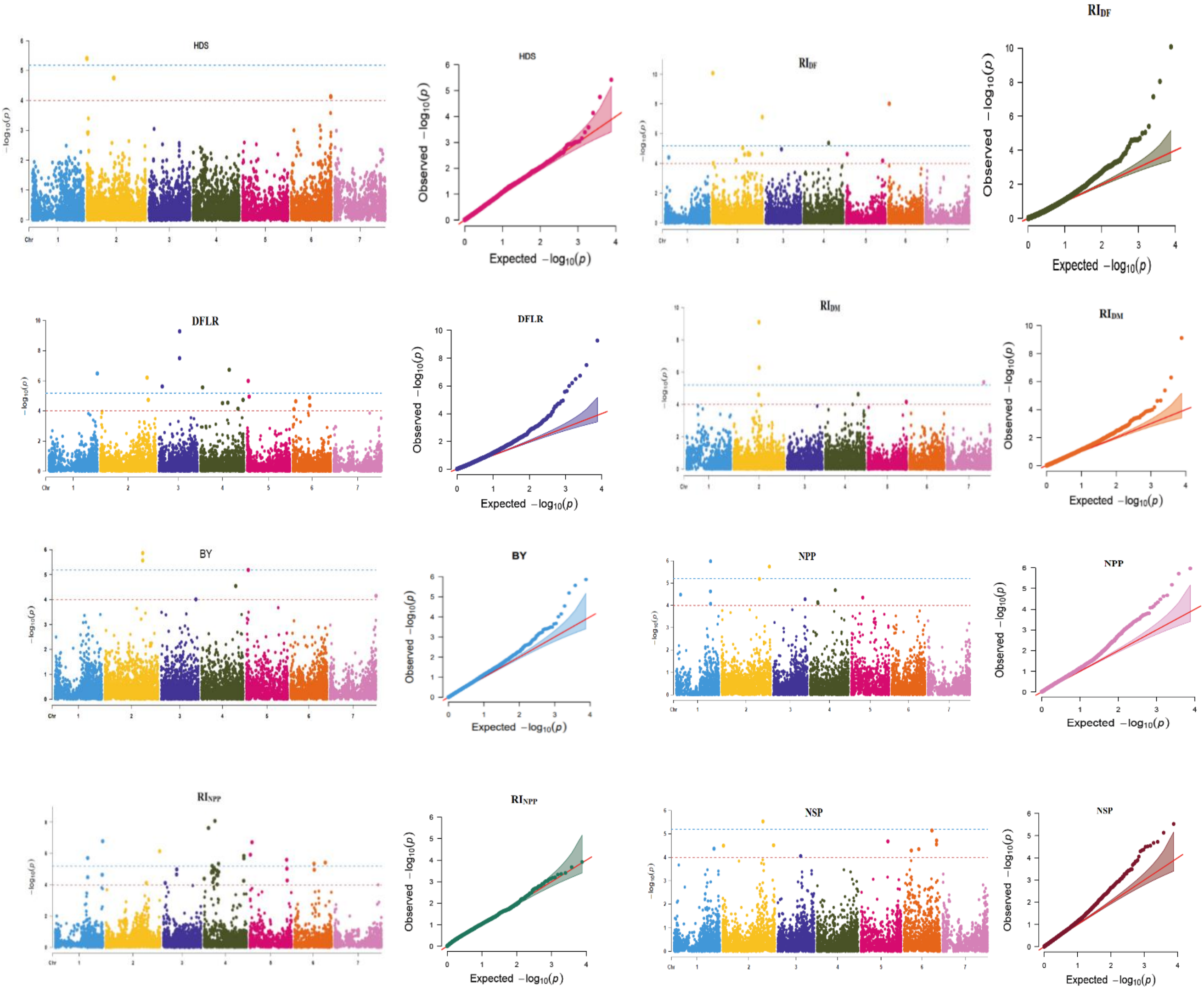

Figure 3. Manhattan plot and QQ plot of the highly significant herbicide tolerance associations existing between the SNP markers of the recorded traits.

3.4. Physical Map and Gene annotation

The physical map presents the SNPs that are located on the genes which are composed of exons (coded regions) and interrupted by introns (non-coding regions) (Figure 4). Out of the eighteen SNPs (A to R) that were found located on the genes, only nine SNPs (A, D, F, H, J, K, O, Q and R) were located on the exomes whereas the rest were found located on the introns on chromosomes 2, 4, 5, 6, and 7.

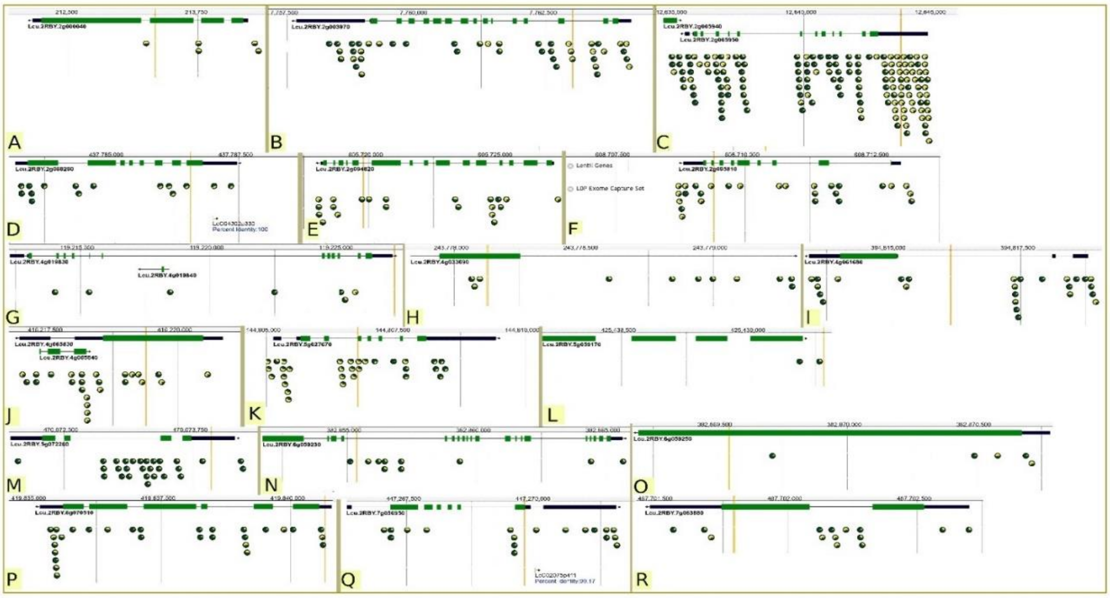

Figure 4. Physical map showing SNP markers location on the genes.

Based on the table of gene annotation, out of the eighteen SNPs that are located inside the gene, four SNPs (AVR-Lc-01885.02-000213238, AVR-Lc-03458.02-007762915, AVR-Lc-03373.02-608709301, and AVR-Lc-10007.07-447269681) were found highly associated with herbicide tolerance (Table 7).

Table 7. Gene annotation table showing the herbicide tolerance SNP marker and the associated gene and their location; in red are the SNPs detected located on the gene.

SNP Code	SNP Name	Marker Location	Gene Location	Gene Name	Description
A	AVR-Lc-01885.02-000213238	Lcu.2RBY.Chr2:213138..213338	Lcu.2RBY.Chr2:212156..214236	Lcu.2RBY.2g000040	Peptide and nitrate transporter type I and II extracellular region ABC transporter related
B	AVR-Lc-03458.02-007762915	Lcu.2RBY.Chr2:7762815..7763015	Lcu.2RBY.Chr2:7757679..7764148	Lcu.2RBY.2g003970	Allantoinase and Dihydroorotase
C	AVR-Lc-01558.02-012643639	Lcu.2RBY.Chr2:12643539..12643739	Lcu.2RBY.Chr2:12635412..12644807	Lcu.2RBY.2g005950	Maternal effect embryo arrest 18 protein
D	AVR-Lc-02719.02-437786468	Lcu.2RBY.Chr2:437786368..437786568	Lcu.2RBY.Chr2:437783190..437787471	Lcu.2RBY.2g068290	Receptor-like kinase
E	AVR-Lc-03341.02-605719692	Lcu.2RBY.Chr2:605719592..605719792	Lcu.2RBY.Chr2:605718142..605727161	Lcu.2RBY.2g094820	Aleurone layer morphogenesis protein
F	AVR-Lc-03373.02-608709301	Lcu.2RBY.Chr2:608709201..608709401	Lcu.2RBY.Chr2:608708810..608713013	Lcu.2RBY.2g095810	Biotin carboxyl carrier acetyl-CoA carboxylase
G	AVR-Lc-05192.04-119227034	Lcu.2RBY.Chr4:119226934..119227134	Lcu.2RBY.Chr4:119212289..119227063	Lcu.2RBY.4g019830	Nitric oxide synthase-associated protein
H	AVR-Lc-05529.04-243778031	Lcu.2RBY.Chr4:243777931..243778131	Lcu.2RBY.Chr4:243777832..243779307	Lcu.2RBY.4g033690	Putative uncharacterized protein
I	AVR-Lc-06116.04-399656942	Lcu.2RBY.Chr4:399656842..399657042	Lcu.2RBY.Chr4:394813417..394818809	Lcu.2RBY.4g061680	DUF1644 family protein

J	AVR-Lc-06170.04-416219290	Lcu.2RBY.Chr4:416219190..416219390	Lcu.2RBY.Chr4:416216947..416220874	Lcu.2RBY.4g065830	F-box plant protein putative
K	AVR-Lc-06798.05-144806665	Lcu.2RBY.Chr5:144806565..144806765	Lcu.2RBY.Chr5:144805139..144809439	Lcu.2RBY.5g027670	AMSH-like ubiquitin thioesterase
L	AVR-Lc-07494.05-425439183	Lcu.2RBY.Chr5:425439083..425439283	Lcu.2RBY.Chr5:425438196..425439199	Lcu.2RBY.5g059170	Eukaryotic translation initiation factor 2c
M	AVR-Lc-07771.05-470073827	Lcu.2RBY.Chr5:470073727..470073927	Lcu.2RBY.Chr5:470071986..470074154	Lcu.2RBY.5g072260	Sterol carrier protein putative
N	AVR-Lc-08869.06-382855264	Lcu.2RBY.Chr6:382855164..382855364	Lcu.2RBY.Chr6:382851739..382865635	Lcu.2RBY.6g059230	PHD zinc finger protein
O	AVR-Lc-08870.06-382869448	Lcu.2RBY.Chr6:382869348..382869548	Lcu.2RBY.Chr6:382869193..382870784	Lcu.2RBY.6g059250	Glycosyltransferase
P	AVR-Lc-09103.06-419840563	Lcu.2RBY.Chr6:419840463..419840663	Lcu.2RBY.Chr6:419835158..419840798	Lcu.2RBY.6g070510	L-fucokinase and GDP-L-fucose pyrophosphorylase
Q	AVR-Lc-10007.07-447269681	Lcu.2RBY.Chr7:447269581..447269781	Lcu.2RBY.Chr7:447266340..447271550	Lcu.2RBY.7g056950	Myelodysplasia-myeloid leukemia factor 1-interacting protein
R	AVR-Lc-10129.07-487701700	Lcu.2RBY.Chr7:487701600..487701800	Lcu.2RBY.Chr7:487701468..487702702	Lcu.2RBY.7g063880	Early nodulin-like protein

Gene annotation showed that SNP AVR-Lc-01885.02-000213238 highly associated with HDS2 (-log10(p) = 6.3) is located on chromosome 2 within a gene annotated Peptide and nitrate transporter type I and II extracellular region ABC transporter related, SNP AVR-Lc-03458.02-007762915 is highly associated with RI_{DF} (-log10(p) = 10.1) is located on chromosome 2 within a gene annotated Allantoinase and Dihydroorotase, SNP AVR-Lc-03373.02-608709301 highly associated with RI_{NP} (-log10(p) = 6.2) and located on chromosome 2 within a gene annotated Biotin carboxyl carrier acetyl-CoA carboxylase, and SNP AVR-Lc-10007.07-447269681 highly associated with RI_{DM} (-log10(p) = 5.4) is located on chromosome 7 within a gene annotated Myelodysplasia-myeloid leukemia factor 1-interacting protein. Nevertheless, only SNPs AVR-Lc-01885.02-000213238, AVR-Lc-03373.02-608709301, and AVR-Lc-10007.07-447269681 were found located on the exomes (coded regions) (Figure 4).

4. Discussion

In Mediterranean environments of cool winters, lentil has slow growth and crop development, which motivates weeds to compete for water, nutrients, sunlight, and space and hosts diseases and pests that causes severe yield losses in this crop (Rubiales and Fernández Aparicio 2012; Sharma *et al.* 2017b). It has been reported that imazethapyr and metribuzin are effective to control weeds when applied to herbicide tolerant lentil accessions. Sources of tolerance to both herbicides were detected in lentils by Balech et al. (2022) and Sharma et al. (2017b, 2018)which allowed them to escape phytotoxicity symptoms caused by the herbicides.

In this study, phytotoxicity symptoms were observed when applying imazethapyr or metribuzin herbicides. The herbicide damage score evaluated the degree of phenotypic phytotoxicity, and considerable variability was observed in the phenotypic response. The recovery or aggravation of the phytotoxicity symptoms is subject to the potential of accessions to metabolize the herbicides and detoxify the plants (Shoup *et al.* 2003). Additionally, the phenology of the tested accessions was also impacted by delaying the flowering and maturity dates of some lentil accessions and caused a reduction in yield and its components. Similar results were obtained in lentil (Sharma *et al.* 2017b, 2018; Balech *et al.* 2022), faba bean (Abou-Khater *et al.* 2021b), and chickpea (Taran *et al.* 2010; Goud *et al.* 2013). Consequently, the phytotoxicity symptoms were ascribed to the inhibition of photosynthesis and plant growth caused by these herbicides as obtained Sharma et al. (2017b).

The phylogenetic tree analysis didn’t discern any specific pattern of genotypes based on their country of origin. Therefore, we suggest the possibility of seed exchange occurrence between countries. Thus, it appears that lentils possess broad genetic diversity that is not particular to a specific geographic location as a result of long-term seed migration and trading across borders.

Limited progress has been made in identifying lentil cultivars tolerant to herbicides through conventional breeding methods, especially that these approaches have been proven to be relatively slow in achieving considerable advances. Hence, it is mandatory to develop genetic markers linked to traits associated with herbicide tolerance in lentils in order to enhance selection accuracy and facilitate early-stage selection. These markers serve as effective tools for selecting adapted and tolerant accessions. Many studies have proved that GWAS is the most successful tool in identifying significant SNPs and candidate genes related to various traits. However, there is a limited number of GWAS reports conducted on lentil such as aphanomyces root rot resistance (Ma *et al.* 2020), prebiotic carbohydrates (Johnson *et al.* 2021), anthracnose resistance (Gela *et al.* 2021), ascochyta blight resistance (Henares *et al.* 2023) and seed protein and amino acids content (Hang *et al.* 2022). Comparing to other crops like maize and sorghum, the development of genetic resources for lentil has been relatively slower. Nevertheless, new horizons in next generation sequencing (NGS) technologies will open as the lentil genome has been recently published (Bett 2016; Kumar *et al.* 2021).

The MetaGWAS method that was applied in the present study, was initially employed in human genetics as it is impossible to gather multi-environmental data for the same population

(Winkler *et al.* 2014; Joukhadar *et al.* 2021). Its effectiveness over standard GWAS analysis was proved, which encouraged its usage in crops (Joukhadar *et al.* 2021; Singh *et al.* 2021). In fact, standard GWAS is more powerful when experiments are conducted under controlled conditions (Zhang *et al.* 2015; Coser *et al.* 2017; Moellers *et al.* 2017). Moreover, conducting experiments in the same environment for a diverse set of accessions that are intended to be grown all over the world can lead to an improper image of the environmental effects on the genetics of the tested set. Many quantitative traits are raw measurements collected from different environments; if standard GWAS analysis is applied, bias effect may be caused which will negatively affect the detection of significant QTL (Chen *et al.* 2010). Therefore, Meta-analysis is an adequate alternative to bypass the previously mentioned challenges of standard GWAS. In our case, MetaGWAS was the best option to be applied since we have an unbalanced set of data collected on 292 accessions, with different treatments on two different locations and two different cropping seasons with a total sample size of 11,956. This approach was also applied by Shook et al. (2021) on a sample of 17,556 accessions of soybean from 73 published studies, by Joukhadar et al. (2021)on a sample of 2,571 accessions of wheat, by Battenfield et al. (2018) on wheat with a total sample size of 4095 and Fikere et al. (2020) on a sample of 585 canola accessions. To the best of our knowledge, this is the first MetaGWAS study applied in lentil crop and targeting QTL associated with herbicide tolerance. Hence, most of the identified QTL in this study appear to be new and have not been reported previously.

Based on the physical map results, four SNPs were detected located on the gene and found highly associated with the recorded traits relative to herbicide tolerance. The associations and mechanisms of tolerance to herbicides between the detected SNPs markers on the genomic regions and the phenotypic traits have been deciphered in the following.

The Peptide and nitrate transporter type I and II extracellular region ABC transporter related protein, belongs to the ATP binding cassette (ABC) transporters family and was detected and found associated with herbicide damage score (HDS). This protein transports amino acids, peptides, and nitrate through the plant’s cell membrane using the energy of ATP hydrolysis (Stacey *et al.* 2002; Pang *et al.* 2012; Lagunas *et al.* 2019). Several studies have proved that plants have the highest diversity of ABC transporters genes such as in Arabidopsis and in rice with 120 and 121 coding sequences respectively (Sánchez-Fernández *et al.* 2001; Song *et al.* 2014). Some of the ABC transporters are responsible for the defence mechanisms to biotic and abiotic stresses and others are involved in the basic functions indispensable for plant growth (Yazaki *et al.* 2009). Furthermore, Van Eerd et al. (2003) acknowledged that this enzyme is typically associated with herbicide metabolism and plant detoxification. Moreover, genes encoding for ABC transporters proteins were also detected in wheat (*Triticum aestivum* L.) (Bhoite *et al.* 2021; Kurya *et al.* 2022), *Arabidopsis thaliana* (Manabe *et al.* 2007), and soybean (Abusteit *et al.* 1985), and performed the function of detoxification of plants from imazethapyr and metribuzin. In this study, the HDS discerned the recovery of some accessions from phytotoxicity symptoms after imazethapyr or metribuzin treatments which might be due to the role of detoxification executed by ABC transporters.

Allantoinase and Dihydroorotase proteins belong to the same superfamily of amidohydrolases (Kim and Kim 1998); they were detected and found highly associated with the RI_{DF}. They participate in various stages of plant development through the *de novo* pathway by using simple molecules such as CO_2, amino acids and tetrahydrofolate to build purine and pyrimidine nucleotides (Moffatt and Ashihara 2002; Dong *et al.* 2019). Imazethapyr and metribuzin have indirect effect on Allantoinase and Dihydroorotase proteins; Imazethapyr disrupts amino acids synthesis and metribuzin (triazine herbicide) inhibits tetrahydrofolate synthesis (Hopfinger 1980). In addition, Kafer (2002) and Duran (2012) reported that flowering stage required the presence of high concentrations of Purine and Pyrimidine. In rice (Wang *et al.* 2023) and in *Arabidopsis thaliana* (Kafer 2002), the genes encoding to purine and pyrimidine metabolism were responsible for the tolerance to the stress that might encounter the plants during the flowering stage. In this study, when either of both herbicides was applied, the flowering stage was delayed for some accessions but not for others. This observation might be explained by the differing concentrations of purine and pyrimidine available in the plants especially during the flowering stage which depends on the lentil variety and its level of tolerance to the applied herbicide.

Biotin carboxyl carrier (BCC) and acetyl-CoA carboxylase proteins (ACC) were detected and found highly associated with RI_{NPP}. BCC is used by the enzyme biotin carboxylase to form carboxybiotin that is transferred to ACC enzyme (ACCase). ACCase engender the carboxylation of acetyl-CoA to form malonyl-CoA; essential for fatty acid synthesis and other secondary compounds such as flavonoids (Capron *et al.* 2009). This enzyme plays an essential role in embryo morphogenesis and in apical meristem development (Capron *et al.* 2009). This explains the detected association with the reduction index of number of pods (RI_{NPP}) in this study much likely as has been reported in *Arabidopsis thaliana* (Baud *et al.* 2003) and *Populus* *simonii* (Chen *et al.* 2013). Moreover, ACCase plays a role in biotic and abiotic stress tolerance in plants. Many studies like in lentil (Bharadwaj *et al.* 2023), *Brassica napus* (Elborough *et al.* 1996; Megha *et al.* 2022), *Arabidopsis thaliana* (Xie *et al.* 2021), and tobacco (Madoka *et al.* 2002) showed that plants can improve their resilience to stress by stimulating the accumulation of ACCase and consequently improving the seed yield. This explains the different levels of tolerance to the applied herbicides expressed in the RI_{NPP}.

Myelodysplasia-myeloid leukemia factor 1-interacting protein was found highly associated with RI_{DM} in this study. It is encoded by (MLF1IP) gene, a transcription factor that was first detected in mammals and *Drosophila* (Wu *et al.* 2021). MLF1IP interacts as a transcriptional repressor with MLF1 and nucleophosmin-MLF1 (NPM-MLF1) to prevent apoptosis (programmed cell death), and thus facilitating cell growth and proliferation in different cell types (Wang and de Vries 2013). As far as we can tell, very rare are the studies that report the presence of MLF1IP in plants and this is the first study that reports its presence in lentil. This gene was also found in tea *Camellia sinensis*, but limited information is available online (A database of gene co-expression network for tea plant (*Camellia sinensis*). Thus, the function of MLF1IP in plants remains to be elucidated, but since they are transcription factors then their role is to regulate cell death triggered by abiotic and biotic stresses (Arce *et al.* 2008; Burke *et al.* 2020).

Moreover, several studies have reported that herbicides cause oxidative stress in plants similar to other abiotic stresses (Tausz 2001; Radwan 2012). This idea highlights the hypothesis that herbicide tolerance in lentil could result from several mechanisms enabling plants to tolerate the stress caused by herbicide treatment very similar to their response to other abiotic stresses. Thus, the tolerance observed in this study is attributed to the mechanisms that significantly contribute to the detoxification of herbicides in lentil crops.

5. Conclusion

Weed management in lentil has become crucial for attempting high yields and good quality to meet the growing global demand. Therefore, the natural genetic variability that lentil crop accessions have shown in previous studies encouraged us for screening a large germplasm collection to search for more powerful and diverse sources for post-emergence herbicide tolerance. This will promote the use of herbicide tolerant varieties with conservation agriculture systems at a lower cost on the farmers. But this method of traditional screening for herbicide tolerance in the field is time consuming, very costly, and hectic. Therefore, genomic selection and marker-assisted selection for herbicide tolerance will greatly improve precision and efficiency of breeding for herbicide tolerance and will help plant breeders in accelerating the breeding process. In this study, we identified four SNP markers that were highly associated with traits related to imazethapyr and metribuzin tolerance using the meta-GWAS method. These identified SNPs could be studied further and used to facilitate selection in breeding programs.

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7. Supplementary Materials

Supplementary Table S1: SNP-trait associations revealed by GWAS analysis; bolded (-Log10) values of SNPs represents the highly significant associations.

QTL	SNP	Chr	allele1	allele0	MAF	HDS2	DF	RI_{DF}	DM	RI_{DM}	PH	BY	SY	NPP	R_{INPP}	NSP
QTL001	AVR-Lc-00233.01-022617381	1	C	T	0.062	1.0	0.1	0.4	5.1	0.1	0.1	0.3	0.1	0.7	0.7	0.5
QTL004	AVR-Lc-00525.01-355542407	1	G	T	0.31	2.0	0.3	0.4	4.1	0.6	0.2	2.2	3.0	0.6	0.8	0.4
QTL004	AVR-Lc-00526.01-355768105	1	G	A	0.306	0.0	1.1	0.1	4.6	0.2	0.3	0.8	0.5	0.3	0.4	1.1
QTL005	AVR-Lc-00556.01-362542743	1	T	C	0.242	1.0	2.3	0.5	4.5	0.6	0.1	1.9	1.8	0.3	0.4	0.3
QTL006	AVR-Lc-00578.01-366320966	1	C	T	0.253	0.8	0.0	0.1	1.5	0.1	0.0	1.7	0.1	2.1	4.5	2.4
QTL006	AVR-Lc-00579.01-366322027	1	A	G	0.136	0.5	0.2	0.1	0.9	0.0	0.1	0.7	0.1	1.0	5.7	0.8
QTL007	AVR-Lc-00831.01-430032156	1	T	C	0.051	0.2	0.2	2.2	0.0	0.3	1.0	0.3	0.2	4.6	0.1	2.1
QTL007	AVR-Lc-00835.01-430931278	1	A	G	0.062	0.1	0.6	1.5	0.2	1.1	0.1	2.5	0.4	6.0	0.0	2.9
QTL007	AVR-Lc-00836.01-431094892	1	C	T	0.071	0.2	0.8	0.5	0.2	0.4	0.5	1.2	0.3	4.1	0.1	1.5
QTL002	AVR-Lc-00911.01-044349219	1	T	C	0.335	1.0	0.9	4.4	0.9	0.3	0.6	0.7	1.3	0.1	0.3	0.4
QTL008	AVR-Lc-00928.01-446895597	1	A	G	0.115	0.0	0.8	0.7	0.3	1.8	0.9	0.6	4.9	0.5	0.0	0.7
QTL009	AVR-Lc-01006.01-468700401	1	C	T	0.111	0.1	1.5	0.4	1.5	0.0	0.4	0.3	0.7	1.9	0.0	4.4
QTL010	AVR-Lc-01313.01-530708051	1	C	T	0.058	0.2	0.0	0.1	1.0	0.0	0.0	0.0	0.7	0.1	4.6	0.0
QTL011	AVR-Lc-01352.01-535793448	1	G	A	0.142	0.5	0.6	0.7	0.6	0.2	0.6	0.5	1.1	0.7	6.8	0.1
QTL012	AVR-Lc-01367.01-537371482	1	C	A	0.077	0.0	6.5	1.7	1.5	1.4	2.2	0.1	0.3	0.0	0.1	0.3
QTL003	AVR-Lc-01375.01-054201950	1	G	A	0.054	1.4	0.6	0.3	0.4	0.3	0.3	1.7	0.4	4.5	0.2	3.7
QTL015	AVR-Lc-01558.02-012643639	2	T	C	0.114	0.5	3.1	4.0	0.1	0.0	0.5	0.7	0.2	0.8	0.3	0.6
QTL016	AVR-Lc-01636.02-014499828	2	C	T	0.383	4.2	0.4	0.9	0.6	0.2	0.9	1.0	0.1	1.7	0.5	0.9
QTL013	AVR-Lc-01885.02-000213238	2	C	T	0.409	6.3	0.8	1.2	0.5	0.1	0.7	1.6	0.4	2.4	0.6	1.0
QTL017	AVR-Lc-02107.02-281092190	2	C	T	0.197	4.8	0.1	0.6	0.2	0.2	0.1	1.2	0.2	1.5	0.3	0.8
QTL018	AVR-Lc-02134.02-290328617	2	C	A	0.075	0.8	0.3	4.2	0.6	0.0	0.2	0.2	0.5	0.5	0.3	0.8
QTL019	AVR-Lc-02169.02-302897953	2	A	G	0.134	0.1	0.1	0.6	0.3	4.6	0.1	0.0	0.1	0.0	0.1	0.3

QTL019	AVR-Lc-02189.02-307011079	2	A	G	0.104	0.1	0.3	0.1	1.0	9.1	1.1	0.1	0.1	0.0	0.0	0.3
QTL019	AVR-Lc-02200.02-309350505	2	C	T	0.13	0.2	0.3	0.3	1.0	6.3	1.4	0.4	0.5	0.0	0.1	0.0
QTL020	AVR-Lc-02445.02-370911708	2	G	A	0.078	0.7	0.3	5.0	1.0	0.0	0.3	0.5	0.7	0.8	0.1	0.7
QTL020	AVR-Lc-02544.02-393755630	2	C	T	0.134	0.2	0.2	4.6	0.3	0.4	0.3	0.3	0.3	0.2	0.8	1.0
QTL021	AVR-Lc-02714.02-436766259	2	G	A	0.071	0.4	1.0	0.2	0.8	0.5	0.4	5.6	1.3	2.7	0.1	1.5
QTL021	AVR-Lc-02715.02-436994699	2	G	A	0.084	0.8	0.5	0.3	0.5	0.3	0.8	5.9	1.6	3.4	0.0	2.8
QTL022	AVR-Lc-02719.02-437786468	2	T	G	0.395	2.0	0.7	2.0	4.0	0.1	0.3	0.7	0.5	0.2	2.6	0.2
QTL023	AVR-Lc-02723.02-439149768	2	A	C	0.21	3.4	1.1	4.6	1.1	0.3	0.0	1.5	0.8	2.1	3.3	2.6
QTL023	AVR-Lc-02725.02-439730005	2	T	G	0.378	2.3	2.2	4.7	2.0	0.8	0.0	0.7	0.3	2.0	2.0	2.4
QTL024	AVR-Lc-02786.02-456774445	2	A	G	0.089	0.9	1.8	4.6	0.3	0.8	0.5	0.1	0.6	1.0	4.1	0.1
QTL025	AVR-Lc-02857.02-473502035	2	A	G	0.133	0.6	2.0	0.6	1.9	0.9	0.3	1.1	0.3	5.2	0.1	5.5
QTL026	AVR-Lc-02988.02-518425731	2	G	T	0.1	0.7	6.2	3.1	0.6	0.2	0.4	0.0	0.9	0.2	0.0	0.5
QTL027	AVR-Lc-03032.02-530710201	2	C	T	0.057	0.1	4.7	0.5	0.0	0.1	0.4	0.1	0.3	0.1	0.0	0.1
QTL028	AVR-Lc-03076.02-539961420	2	G	A	0.246	0.3	3.1	0.9	4.5	0.6	0.4	0.5	0.9	1.3	0.3	0.4
QTL029	AVR-Lc-03296.02-599856144	2	T	G	0.089	0.7	1.2	1.3	0.2	0.9	0.5	1.5	0.7	5.7	0.0	4.5
QTL030	AVR-Lc-03341.02-605719692	2	G	A	0.351	0.4	0.2	4.6	0.4	1.7	0.1	0.1	0.5	0.5	0.1	1.2
QTL031	AVR-Lc-03373.02-608709301	2	A	G	0.092	0.6	0.7	1.2	0.6	0.3	0.2	0.1	0.1	0.6	6.2	0.6
QTL032	AVR-Lc-03379.02-609257610	2	T	C	0.085	0.1	2.5	7.1	0.5	0.9	2.2	0.0	1.7	0.0	0.0	0.5
QTL014	AVR-Lc-03458.02-007762915	2	C	T	0.05	0.8	3.6	10.1	0.6	2.8	0.0	1.3	0.3	3.4	0.2	4.5
QTL037	AVR-Lc-03698.03-152405578	3	A	G	0.057	0.4	0.6	0.0	1.6	0.3	0.1	0.7	0.7	0.6	4.7	0.0
QTL037	AVR-Lc-03712.03-154837989	3	G	A	0.064	0.3	0.0	0.0	1.4	0.3	0.1	1.4	0.8	0.5	5.0	0.1
QTL038	AVR-Lc-03869.03-192934047	3	G	A	0.489	0.7	1.5	5.0	0.1	0.9	1.5	0.9	1.5	0.0	0.4	0.2
QTL039	AVR-Lc-03983.03-230295656	3	C	T	0.074	0.1	7.5	0.4	1.3	0.0	1.0	0.2	0.6	0.0	0.1	0.2
QTL039	AVR-Lc-03987.03-231578053	3	C	A	0.074	0.1	9.3	0.6	1.3	0.2	1.1	1.0	0.5	2.2	0.1	1.9

QTL033	AVR-Lc-04030.03-023938415	3	G	A	0.058	0.4	0.7	0.1	0.3	0.6	0.8	0.4	2.2	0.1	4.1	0.1
QTL040	AVR-Lc-04137.03-269539529	3	C	A	0.071	0.2	2.5	0.5	1.4	0.3	0.3	0.7	0.4	1.2	0.1	4.1
QTL034	AVR-Lc-04656.03-038315503	3	A	G	0.12	0.2	5.6	2.7	1.4	1.5	0.6	0.7	0.3	0.3	0.7	0.5
QTL041	AVR-Lc-04751.03-400242285	3	G	A	0.225	1.5	1.5	0.6	0.4	2.6	2.4	4.0	0.9	4.3	0.6	3.4
QTL042	AVR-Lc-04888.03-426261315	3	T	C	0.093	0.1	0.4	0.5	0.0	0.2	4.6	0.1	0.1	0.2	0.3	0.4
QTL035	AVR-Lc-05053.03-072044999	3	G	A	0.382	0.1	0.8	0.1	4.2	0.1	0.2	0.4	1.6	0.3	1.5	0.2
QTL036	AVR-Lc-05067.03-074793162	3	A	G	0.191	0.1	0.0	0.1	1.6	0.1	0.8	1.5	4.7	0.2	0.1	0.1
QTL036	AVR-Lc-05096.03-082600182	3	C	T	0.191	0.1	0.0	0.0	1.5	0.0	0.6	1.4	4.1	0.0	0.1	0.1
QTL047	AVR-Lc-05145.04-104990751	4	G	A	0.091	0.8	0.6	0.9	1.0	0.2	0.1	0.8	0.1	0.5	4.8	0.2
QTL047	AVR-Lc-05152.04-107024094	4	G	A	0.081	0.1	0.0	0.3	0.3	0.7	0.0	0.2	0.0	0.1	5.1	0.2
QTL047	AVR-Lc-05161.04-109440416	4	G	A	0.092	0.2	0.2	0.5	0.4	0.4	0.1	0.3	0.1	0.2	4.4	0.1
QTL047	AVR-Lc-05167.04-112177418	4	T	C	0.109	0.8	0.9	2.5	0.3	1.2	0.5	1.1	0.1	1.1	4.4	0.3
QTL047	AVR-Lc-05182.04-116949401	4	C	T	0.094	0.7	0.4	0.9	0.7	0.3	0.1	0.7	0.1	0.5	4.4	0.2
QTL047	AVR-Lc-05192.04-119227034	4	T	C	0.084	1.0	0.6	0.9	0.8	0.4	0.1	0.6	0.1	0.6	5.0	0.3
QTL047	AVR-Lc-05203.04-122734802	4	C	T	0.063	0.4	1.3	2.7	0.3	0.5	0.1	1.5	0.1	0.5	8.1	0.8
QTL047	AVR-Lc-05259.04-144601100	4	T	C	0.063	0.2	0.0	0.4	1.1	0.4	0.2	0.2	0.2	0.1	4.6	0.5
QTL047	AVR-Lc-05266.04-145430883	4	A	G	0.103	1.0	0.6	0.7	0.7	0.5	0.3	1.1	0.0	1.4	4.9	1.4
QTL047	AVR-Lc-05278.04-148771802	4	C	T	0.102	0.2	0.0	0.1	0.6	1.0	0.1	0.3	0.1	0.7	4.0	1.7
QTL047	AVR-Lc-05287.04-150464062	4	T	C	0.087	0.4	0.4	0.6	0.8	0.6	0.3	0.2	0.0	0.2	4.6	0.1
QTL047	AVR-Lc-05296.04-152490575	4	T	C	0.089	0.2	0.1	0.1	0.7	0.2	0.2	0.2	0.1	0.1	4.9	0.2
QTL047	AVR-Lc-05305.04-155525631	4	T	C	0.087	0.4	0.2	0.5	0.6	0.6	0.2	0.2	0.0	0.2	4.6	0.1
QTL047	AVR-Lc-05322.04-163251061	4	G	A	0.08	0.4	0.1	0.4	0.9	0.3	0.0	1.0	0.2	0.4	5.4	0.3
QTL047	AVR-Lc-05328.04-165504278	4	C	T	0.088	0.3	0.3	0.6	0.4	0.3	0.2	0.2	0.1	0.2	4.8	0.2
QTL043	AVR-Lc-05340.04-001679490	4	T	C	0.078	1.7	0.3	0.0	0.2	0.1	1.0	0.5	0.1	1.1	4.4	0.8

QTL044	AVR-Lc-05454.04-021398009	4	C	T	0.056	0.3	5.6	0.4	3.2	0.7	0.4	0.4	0.2	0.5	0.0	0.3
QTL048	AVR-Lc-05529.04-243778031	4	C	T	0.438	0.5	4.5	2.4	0.3	2.5	0.2	0.5	0.7	0.2	0.5	0.0
QTL049	AVR-Lc-05740.04-302184757	4	G	A	0.073	0.3	4.5	5.4	1.1	1.4	0.3	0.4	0.6	0.4	0.1	0.1
QTL050	AVR-Lc-05789.04-315309984	4	G	A	0.059	0.6	0.5	0.4	0.4	0.0	0.1	1.6	1.3	4.7	0.2	3.5
QTL051	AVR-Lc-05801.04-318079189	4	G	A	0.072	0.1	6.7	0.1	2.9	1.1	0.9	1.0	0.7	0.8	0.0	1.1
QTL052	AVR-Lc-06098.04-394816044	4	G	T	0.054	1.0	0.3	0.3	0.9	0.1	0.8	4.5	0.9	2.4	0.2	0.7
QTL053	AVR-Lc-06116.04-399656942	4	G	T	0.183	0.2	0.2	0.2	1.2	4.6	0.0	0.5	0.5	0.4	0.2	0.0
QTL054	AVR-Lc-06170.04-416219290	4	C	A	0.116	0.3	4.1	0.1	0.8	1.7	0.8	0.1	0.1	0.4	0.1	0.6
QTL055	AVR-Lc-06265.04-438198992	4	C	T	0.417	1.5	0.7	0.4	4.1	0.8	1.6	0.3	0.6	0.1	0.3	0.2
QTL056	AVR-Lc-06326.04-449185572	4	C	A	0.111	1.0	0.0	0.2	0.4	0.2	0.5	1.0	1.0	0.3	4.3	0.7
QTL057	AVR-Lc-06339.04-451674618	4	C	A	0.071	0.2	0.3	0.9	0.1	0.2	0.4	0.9	0.9	0.2	5.8	0.6
QTL057	AVR-Lc-06341.04-451714023	4	G	T	0.062	0.2	0.3	0.2	0.2	0.2	0.3	1.4	0.3	0.1	5.7	0.5
QTL058	AVR-Lc-06433.04-469775636	4	G	A	0.355	0.2	1.0	0.5	4.3	3.6	0.1	0.4	0.6	0.5	0.3	1.2
QTL059	AVR-Lc-06444.04-471058793	4	G	A	0.127	0.5	4.7	1.2	2.4	0.1	0.3	0.5	0.1	0.1	0.0	0.1
QTL045	AVR-Lc-06527.04-050552783	4	A	C	0.094	0.9	0.0	0.2	0.1	0.1	0.3	0.4	0.8	0.7	7.6	0.3
QTL047	AVR-Lc-06633.04-084622211	4	G	A	0.078	0.1	0.0	0.0	0.1	0.6	0.1	0.4	0.0	0.2	4.8	0.2
QTL047	AVR-Lc-06644.04-089591157	4	G	A	0.092	1.0	0.4	0.9	0.7	0.1	0.1	1.3	0.1	0.8	4.9	0.4
QTL047	AVR-Lc-06652.04-092451825	4	T	C	0.08	1.6	0.6	1.0	0.8	0.5	0.1	1.4	0.2	1.5	5.2	0.6
QTL046	AVR-Lc-06657.04-094813562	4	G	A	0.303	1.3	0.4	0.8	0.0	0.1	0.6	1.2	0.1	4.1	0.4	0.8
QTL047	AVR-Lc-06659.04-095095176	4	A	G	0.084	0.4	0.2	0.8	0.2	0.6	0.4	0.4	0.0	0.6	4.8	0.1
QTL046	AVR-Lc-06666.04-097561437	4	T	C	0.448	1.7	0.2	0.1	0.1	0.4	1.1	1.5	0.1	4.1	1.2	1.3
QTL047	AVR-Lc-06671.04-099280552	4	A	G	0.094	0.4	0.1	0.1	0.9	0.1	0.3	0.3	0.1	0.2	4.2	0.5
QTL061	AVR-Lc-06725.05-011593595	5	C	T	0.09	0.2	6.0	4.6	0.4	0.1	0.2	0.2	0.7	1.9	0.1	0.5
QTL065	AVR-Lc-06798.05-144806665	5	C	T	0.389	2.8	0.3	0.3	0.5	0.9	0.4	1.4	0.3	4.3	0.3	3.1

QTL062	AVR-Lc-06969.05-022095933	5	A	C	0.071	0.4	0.1	1.9	0.7	2.2	0.4	5.2	0.9	2.7	0.2	1.0
QTL063	AVR-Lc-06971.05-022411138	5	A	C	0.091	0.0	4.9	0.1	0.3	0.5	0.4	0.1	0.5	0.0	0.1	0.1
QTL064	AVR-Lc-07086.05-026835995	5	T	G	0.06	0.0	0.1	0.2	0.2	0.0	0.7	0.7	0.7	0.3	6.7	0.1
QTL066	AVR-Lc-07199.05-317998176	5	C	A	0.117	0.2	1.6	0.5	0.2	0.1	0.6	0.1	0.0	0.6	0.1	4.7
QTL067	AVR-Lc-07474.05-420462935	5	C	T	0.071	0.4	0.1	0.1	0.2	0.7	0.6	0.5	0.6	0.3	5.6	0.6
QTL067	AVR-Lc-07483.05-422569016	5	G	A	0.075	0.1	0.1	0.1	0.6	0.8	0.6	0.3	0.2	0.0	5.0	0.5
QTL067	AVR-Lc-07494.05-425439183	5	G	T	0.128	0.6	0.1	0.5	0.4	0.3	0.4	1.5	0.3	1.3	4.3	0.7
QTL068	AVR-Lc-07604.05-441955698	5	G	A	0.091	0.8	0.5	4.2	2.1	0.6	0.3	0.7	0.5	0.7	0.1	1.1
QTL069	AVR-Lc-07771.05-470073827	5	C	T	0.054	0.0	0.9	2.7	0.1	4.2	0.4	0.6	0.1	1.4	0.3	0.9
QTL060	AVR-Lc-07900.05-008235645	5	A	C	0.089	0.9	0.2	0.7	0.7	0.7	0.0	0.4	0.1	0.0	5.9	0.2
QTL070	AVR-Lc-08000.06-011687720	6	T	G	0.456	0.2	4.1	2.8	0.2	0.3	0.1	0.1	0.5	0.2	0.4	0.8
QTL071	AVR-Lc-08010.06-011899372	6	C	T	0.053	0.1	3.1	8.0	0.9	0.8	0.8	0.7	0.1	1.2	0.0	2.0
QTL074	AVR-Lc-08166.06-172835867	6	C	A	0.056	0.0	0.6	1.6	0.9	0.7	0.4	0.5	0.1	1.4	0.1	4.3
QTL075	AVR-Lc-08209.06-184525439	6	T	C	0.061	0.1	4.9	0.4	0.3	2.1	0.5	0.2	0.2	0.2	0.1	0.1
QTL075	AVR-Lc-08213.06-186928146	6	C	T	0.075	0.1	4.4	0.4	0.3	2.1	0.4	0.3	0.5	0.1	0.1	0.1
QTL076	AVR-Lc-08292.06-221642340	6	C	T	0.07	0.3	0.3	0.4	1.0	0.1	0.4	0.2	0.0	0.4	5.4	1.3
QTL076	AVR-Lc-08300.06-223824687	6	G	A	0.078	0.2	0.2	0.3	1.1	0.0	0.4	0.1	0.0	0.3	5.0	1.1
QTL072	AVR-Lc-08587.06-031922562	6	G	A	0.094	0.7	4.6	0.1	0.1	0.5	0.6	0.6	0.1	2.8	0.3	1.1
QTL078	AVR-Lc-08637.06-328113285	6	C	T	0.093	0.6	1.4	0.1	0.9	1.2	0.1	0.7	0.2	2.6	0.2	5.1
QTL077	AVR-Lc-08717.06-348699147	6	G	A	0.077	0.3	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.9	5.4	0.1
QTL078	AVR-Lc-08863.06-381679296	6	T	G	0.162	0.3	0.9	1.0	0.3	0.0	0.0	0.6	0.4	0.9	0.0	4.5
QTL078	AVR-Lc-08869.06-382855264	6	A	G	0.153	0.3	1.1	1.3	1.2	0.0	0.0	0.2	0.5	1.0	0.1	4.7
QTL079	AVR-Lc-08870.06-382869448	6	G	A	0.345	0.2	1.0	1.5	5.0	0.5	0.3	0.4	0.1	0.2	0.3	1.9
QTL080	AVR-Lc-09049.06-413154045	6	G	A	0.226	4.2	0.7	0.1	0.1	0.2	0.4	1.0	0.6	0.5	0.4	0.3

QTL080	AVR-Lc-09063.06-414723087	6	A	G	0.166	4.5	0.8	0.3	0.6	0.3	0.6	0.5	1.5	1.4	0.5	0.6
QTL081	AVR-Lc-09086.06-418350448	6	G	A	0.053	1.0	0.3	1.8	4.3	0.1	0.2	0.4	0.9	0.6	0.2	0.5
QTL082	AVR-Lc-09103.06-419840563	6	A	G	0.188	0.8	0.3	0.2	4.3	0.2	0.2	0.5	1.2	0.1	0.2	0.3
QTL073	AVR-Lc-09234.06-084823796	6	A	G	0.419	0.7	1.4	0.1	0.8	0.4	0.2	0.9	0.3	1.8	0.2	4.3
QTL083	AVR-Lc-10007.07-447269681	7	C	T	0.368	0.6	0.2	0.7	0.4	5.4	0.4	0.6	0.5	0.3	0.3	0.4
QTL084	AVR-Lc-10129.07-487701700	7	A	C	0.334	0.6	0.0	0.1	4.4	0.4	0.4	0.4	2.0	0.3	0.3	1.0
QTL085	AVR-Lc-10391.07-528906358	7	G	A	0.469	0.5	0.3	0.1	0.6	1.7	0.1	4.1	0.6	0.4	1.1	0.4

QTL: quantitative trait loci, SNP: single nucleotide polymorphism, Chr: Chromosome, MAF: minor allele frequency, HDS2: second herbicide damage score, DF: days to flowering, RI_{DF}: DF reduction index, DM: Days to maturity, RI_{DM}: DM reduction index, PH: Plant height, BY: Biological yield per plant, SY: seed yield per plant, NPP: number of pods per plant, RI_{NPP}: NPP reduction index, NSP: number of seeds per plant.

Chapter V
General Conclusions

- A wide range of variability was found in the response of lentil accessions towards the post-emergence application of imazethapyr or metribuzin where different degrees of phytotoxicity were observed (Chapter II).

- The post-emergence application of imazethapyr or metribuzin caused a significant delay in flowering time and maturation, and significant reduction of plant height (Chapter II).

- The selection of herbicide tolerant varieties by using Herbicide Damage score (HDS) was found to be a good tool for selection in preliminary stages of screening; but selection via the reduction index (RI_{SY}) was found to be the more effective (Chapter II).

- Four accessions were identified independently tolerant to metribuzin and imazethapyr; IG4400 and IG323 were found adapted to high rainfall environments and IG5722 and IG4605 were adapted to low rainfall environments (Chapter II).

- The yield and stability of performance across environments are the most effective indicators for a successful selection of herbicide tolerant varieties (Chapter III).

- The usage of a combination of stability parameters when evaluating the performance of a group of accessions is mandatory for a better assessment across environments (Chapter III).

- Environments that are less likely to encounter drought periods are more recommended to conduct herbicide screening trials (Chapter III).

- GGE biplot of SY identified IG70056 (38) as a superior line with high and stable yield across years and locations with tolerance to imazethapyr and metribuzin (Chapter III).

- IG4605 (19), IG195 (6) and IG156635 (12) were found specifically adapted to one mega environment (Chapter III).

- Crossing herbicide tolerant genotypes with stable performance with genotypes adapted to specific environments is recommended to develop superior herbicide tolerant genotypes that are adapted to various mega environments (Chapter III).

- The Meta-GWAS analysis showed that out of the 125 SNP associated with phenological and yield traits under herbicide tolerance, 36 SNPs were found highly significant (Chapter IV).

- SNPs from gene annotated Peptide/nitrate transporter type I/II extracellular region ABC transporter related, Allantoinase / Dihydroorotase, Biotin carboxyl carrier acetyl-CoA carboxylase, and Myelodysplasia-myeloid leukemia factor 1-interacting protein were found highly significantly associated with herbicide tolerance traits (Chapter IV).

- Marker assisted selection programs could use these findings to enhance herbicide tolerance in lentil crop (Chapter IV).