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From Petri dish to field: testing Greek lentil accessions for imazamox tolerance

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Abstract

This work aimed to study the intrinsic tolerance of Greek lentil accessions to imazamox herbicide by combining bioassays, pot and field experiments. Initially, 31 genotypes were evaluated in Petri dish bioassays for their tolerance to six concentrations of imazamox. The average root length of 10 lentil seedlings/dish at seven days after herbicide application was used for non-linear regression analysis and the GR_{50} values (the amount of the herbicide required for 50% root length reduction of the seedlings) were estimated to calculate the resistance ratio (R/S) of each cultivar. The results of the *in vitro* test clued the selection of nine accessions for further study in pot experiment, to assess their tolerance to four rates [0 (control), 20, 30, 40 g ai ha^{-1}] of imazamox post-emergently applied at the seven true-leaf stage (V7 stage). Five weeks after treatment, the number of survived plants was recorded and the above-ground dry weight was determined in each pot. There was no direct correlation in the results of *in vitro* test and the pot experiment, suggesting no matching between the two methods. The evaluation of five accessions (cultivars with high commercial interest and accessions sporting tolerance in pot experiment) in field experiment demonstrated different but increased susceptibility to imazamox. Specifically, compared to the untreated control, the imazamox treatments reduced plant growth, delayed flowering and maturity and reduced yield, dry weight, 1000-seed weight and harvest index. Yet the protein concentration was increased in herbicide treatments. The findings of the study showed clearly that the evaluated lentil accessions lack genes with resistance to imazamox and different methods have to be used for assessing any potential tolerance.

Keywords: ALS; Imazamox; Lentil; Post-emergence application.

Introduction

Lentil (*Lens culinaris* Medik.) is one of the most important legumes worldwide and is grown under both irrigated and rain-fed conditions in most regions of the world (Rasheed et al., 2010). Despite its importance for human nutrition, the expansion of the crop is restricted, amongst others, by weed interference. Lentil is a poor competitor against weeds and this is attributed to the short plant stature and the slow early growth (Ball et al., 1997; Sarker and Erskine, 2006). This implies significant yield losses in case of non-effective weed control.

In general, weeds compete with crops mainly for space, light, soil water, nutrients and thus reduce the quantity and degrade the quality of lentil (Dangwal et al., 2010). Yield reduction by weeds varies and depends on the type, density and distribution of weeds in the field, but also by the agronomic practices implemented in each area. Lentil yield losses by weeds have been estimated to as high as 84% in West Asia (Sarker and Kumar, 2011). Apart from direct loss of production, ineffective weed control indirectly affects the quality of the product. When weeds grow and develop during the maturing, usually compete weakly the lentil (McDonald et al., 2007). However, if the crop hosts, during harvest, a large number of green, immature weeds and/or weed seeds can cause post-harvest problems such as fungal diseases and discoloration of seeds (Matus et al., 1993).

Chemical control is the most convenient and cost-effective method of suppressing the weeds in lentil (Yasin et al., 1995). Especially for broad-leaved and parasitic weeds, their control is a major agronomic issue in most of the legume crops due to the absence of selective herbicides (Beckie et al., 2006; Yenish et al., 2009). Herbicides of dinitroanilines (such as trifluralin and pendemethalin), triazinones (such as metribuzin), chloroacetamide (such as metolachlor) and imidazolinones (such as imazethapyr) have been evaluated for possible use in lentil (Wall and McMullan, 1994; Wall, 1996; Erman et al., 2004, Elkoca et al., 2005). However, these herbicides can be phytotoxic, cannot combine with graminicides and in order to prevent injuries, most of them, should be applied early in the growing season, which does not coincide with the optimal weed control timing (McVicar et al., 2006). The critical period for weed control (CPWC), the time window in the growth cycle of the crop during which weeds must be controlled to avoid yield losses (Van Acker et al., 1993), is a tool to determine the optimal timing for weed control. Therefore, to avoid yield losses, weeds should be controlled at the beginning of the CPWC and their control should be sustained till the end of the growing season (Fedoruk et al., 2011). CPWC has contributed to the determination of the application time of post-emergence herbicides and found to vary by region, sowing season, agronomic practices and climatic conditions. CPWC for lentil under Mediterranean conditions was found to be 7-13 weeks after sowing (Al-Thahabi et al., 1994).

Considering the above-mentioned and the high cost of a new active herbicide ingredient to be discovered, to address poor broadleaf weed control in lentil, cultivars with imidazolinone (IMI) tolerance have been developed by breeders at the University of Saskatchewan Crop Development Centre, Canada (Tan et al., 2005; Slinkard et al., 2007). Imidazolinones inhibits acetolactate synthase or acetohydroxyacid synthase (ALS or AHAS), which is a key enzyme in the biosynthesis of branched-chain amino acids in plants, valine, leucine and isoleucine. The IMI herbicides have a wide spectrum of weed control activity, including grass, broadleaf and parasitic weeds, low usage rates, low mammalian toxicity (Rainbolt et al., 2009) and commonly used in other IMI-tolerant crops such as maize, rise, sunflower, wheat and oilseed rape (Tan et al., 2005). Recently, Vasilakoglou et al. (2013) reported tolerance of common vetch (*Vicia sativa* L.) and red pea (*Lathyrus cicera* L.) to imazamox testing Greek genetic material. Furthermore, the IMI herbicide application period potentially is longer, because it can be applied to lentil until the 11-node stage without crop injury (Fedoruk and Shirtliffe, 2011). Tolerance to IMI herbicides is derived from mutagenesis induced amino-acid substitutions (Tan et al., 2005) while natural tolerance to IMI-herbicides lies in the ability to rapidly metabolize the herbicide (Vencill et al., 2012).

This work was triggered by the great difficulty Greek lentil growers face to control broadleaf weeds. The problem has become more acute after the withdrawal of main herbicides (alachlor and prometryn) and concurrently the absence of selective post-emergence herbicides. Therefore, the aim of this work was to evaluate the Greek lentil accessions for putative existence of useful genetic variability to tolerate imazamox, a member of IMI family. This study evaluated the *in vitro* as well as the *in vivo* response to imazamox in order to test for tolerant gene or tolerance due to the metabolism.

Materials and Methods

Seed source

Thirty-one accessions (cultivars and landraces) of lentil (*Lens culinaris* Medik.) bred by conventional breeding and deposited at ELGO-"Demeter", Institute of Industrial and Fodder Plants, Larissa, Greece were used in this study. Specifically, the genetic material originated from Greece (14 accessions), ICARDA, Algeria, Bulgaria, USA, India, Jordan, Morocco, Turkey and Chile.

In vitro screening procedure

Lentil accessions were initially evaluated in Petri dish bioassays for tolerance to six concentrations of imazamox [0 (control), 0.01, 0.1, 1, 10, 100 mg ai imazamox L^{-1}]. In each 9-cm Petri dish, 10 seeds of each accession were placed in on double filter paper and delivered 5ml suspension of each concentration of imazamox. The Petri dishes were kept into a dark growth chamber at 25 ^{o}C constantly. Each treatment was triplicated and the test was conducted twice.

Seven days after imazamox application, root length of the 10 seedlings were measured and averaged over each dish. The data of the two tests were pooled together and used to determine the amount of imazamox required for 50% root length reduction of lentil seedlings (GR_{50} values). Root length data were subjected to nonlinear regression analysis using the log-logistic equation proposed by Seefeldt et al. (1995):

$$y = C + \frac{D - C}{1 + \exp\{b[\log(x) - \log(GR50)]\}}$$

where C= the lower limit, D= the upper limit, b= the slope at the GR_{50} and GR50= the herbicide rate required for 50% root length. In this regression equation, the ALS inhibitor rate (g ha^{-1}) was the independent variable (x) and the root length (percentage of the untreated control for each cultivar) was the dependent variable (y). The levels of resistance for all the accessions (R and S) were determined by the resistance ratio (R/S), which was calculated as the GR_{50} of the R-resistant accession divided by the GR_{50} of the S-control accession.

Pot experiment

The results of the *in vitro* test clued the selection of nine accessions (Dimitra, Flip 2003-24L, ILL-590, ILL-6811, Ikaria, 73, 81S 15, Thessaly and Samos) for further study in pot experiment. In 0.9 L pots, on 9 March 2012, eight seeds per pot were sown. The soil was a silty clay loam with pH 8.0, 1.5% organic matter, 40 mg N-NO_3 kg^{-1}, 5 mg P-Olsen kg^{-1} and 190 mg K kg^{-1}. The pots were placed in a glasshouse till germination completion and then were transferred outdoors under a net-protected area. Each pot was considered as a replicate for the subsequent whole-plant experiment performed. Pots received no fertilization and were sub-irrigated to keep soil water at field capacity. Two weeks after emergence, at the 4-leaf stage, thinning left four plants per pot.

Imazamox application was conducted, at the stage of the full expansion of 7-8 leaves, at four rates of imazamox [0 (control), 20, 30, 40 g ai ha^{-1}]. Herbicide was applied with a portable field plot sprayer (Azo-Sprayers, Ede, The Netherlands) using a 2.4 m wide boom fitted with six flat-fan nozzles (Teejet® 8002). The sprayer was calibrated to deliver 300 L ha^{-1} of water at 280 kPa pressure. Five weeks after the treatment, the number of survived plants and the dry weights per pot were recorded and the dry weight per plant was calculated.

Alike the *in vitro* test, the log-logistic equation was used to calculate dry weight resistance ratios (R/S).

Field experiment

During 2012-13 growing season, a field trial, using five lentil accessions (Dimitra, Flip 2003-24L, Ikaria, Thessaly and Samos) was established at the farm of Aristotle University of Thessaloniki (40° 32′ 12 N, 22° 59′ 21 E, 6 m) on 1-2 December 2012. Temperature and precipitation during the experimental period are shown in Figure 1.

The experimental design was a split-plot with three replications; lentil accessions were in the main plots and imazamox rates at the subplots. Each subplot was consisted of eight rows, 2 m long and at 25 cm separation and the seeding rate was 150 seeds m^{-2}. At the stage of 7-8 leaves, imazamox was applied at four rates [0 (control), 20, 30, 40 g ai ha^{-1}] by adding the surfactant Dash (0.1% v v^{-1}) using the previously mentioned sprayer. No rain occurred two days before or after the application.

At maturity, which varied chronically among lentil accessions and imazamox rates, an area of 0.5 m^2 per subplot was harvested and plants were threshed by hand. Above-ground dry weight (DW), seed yield (SY), 1000-seed weight (TSW) and harvest index (HI) were determined for each subplot. A seed subsample per subplot (~5 g) was dried, at 75 °C till constant weight and ground to fine powder. Seed nitrogen concentration (%N) was measured on an elemental analyzer (EA3000, EuroVector SpA, Milan, Italy), and seed protein concentration (PC) was calculated as the product %N×6.25.

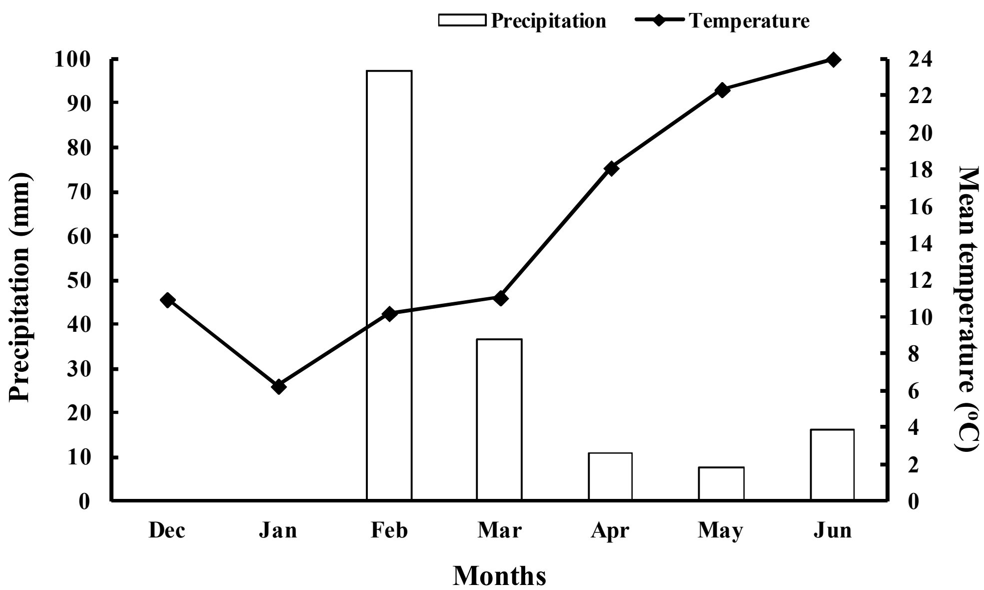

Figure 1. Monthly mean air temperature and precipitation during 2012-2013 growing season. In December 2012 and January 2013 rainfall was nil.

Statistical analysis

Data of the *in vitro* experiment (GR_{50} and R/S ratios) were subjected to analysis of variance (ANOVA) using a factorial approach (6 imazamox rates×31 accessions). Alike, pot experiment R/S ratios were analysed by ANOVA as a 9 accessions×4 imazamox rates factorial approach. Finally, data obtained by the field experiment (DW, SY, TKW, HI and PC) were subjected to a split-plot analysis using a 5 accessions×4 imazamox rates×3 replications factorial arrangement.

Calculations and statistical analyses were performed using the SPSS ver. 17 software package (SPSS Inc., Chicago, IL, USA). Means were compared by least significant difference (LSD) test at the 5% level of significance.

Results and Discussion

Verification of crop cultivar tolerance to a specific herbicide may take an array of tests ranging from Petri dish to field trials. Seed germination bioassays are widely used for distinguishing sensitive and tolerant genotypes in various rates of herbicides and its eminent advantage is that is faster and cheaper compared to other methods. However, bioassays can only detect tolerance due to modification of the herbicide site of action, ALS enzyme tolerant in our case and not due to enhanced metabolism of herbicides, which occurs in later growth stages (Kaundun et al., 2010). For these reasons, it is necessary to confirm any bioassay tolerance by evaluating plants in pots and/or in the field.

In the study, GR_{50} and the concomitant R/S values [GR_{50} (R)/GR_{50} (S)] obtained by the seed bioassays sounded promising for two (Flip 2003-24L and ILL 6811) out of 31 accessions that showed very high R/S values (71 and >184, respectively). On the contrary, a Greek-bred cultivar, Thessaly, was the most susceptible to imazamox (Table 1).

Table 1. Estimated GR_{50} using root lengths and resistance ratios (R/S) of the 31 lentil accessions in the *in vitro* experiment.

Accession	GR_{50}	R/S	Accession	GR_{50}	R/S
Dimitra	5.1	8.5	F-86	14.0	22.0
Flip 92-36L	1.5	2.5	ILL-590	7.5	11.8
Flip 94-5L	7.0	11.7	ILL-6811	117.0	184.0
Flip 2002-1L	5.1	8.0	ILL-7698	4.9	7.7
Flip 2003-12L	6.6	10.4	LL-35	6.3	9.9
Flip 2003-24L	45.5	71.5	Ikaria	2.8	4.4
Flip 2003-50L	17.9	28.1	Athena	6.0	9.4
Flip 2003-57L	4.4	6.9	Artemis	11.2	17.5
M-15305	15.0	23.6	73	7.7	12.2
M-17003	2.9	4.6	33-032-10403	2.6	4.1
ILL-96	0.7	1.2	HC-125	6.4	10.0
F-81	1.4	2.2	81S 15	9.3	14.7
F-82	3.8	6.0	LC-960254	2.2	3.4
F-83	6.0	9.4	Limnos	2.6	4.1
F-85	1.7	2.7	Thessaly (S)	0.6	1.0
			Samos	6.2	9.7

The above findings were the clue for the next step, where the cultivars with high commercial interest and accessions sporting tolerance in bioassays were selected to be tested in pots. Data analysis of the dry weights showed that the growth of plants was affected by genotype and the application rates, while the pertinent interaction was not significant. However, as Figure 2 shows, at the recommended rate of imazamox (1×), ILL-6811, Samos and Flip 2003-24L sustained their growth achieving dry weights as high as 80.7%, 79.7% and 63.7%, respectively, compared to the control. For the rest of the accessions, the recommended rate of imazamox halved the dry weights compared to the control, with cv Thessaly to show the highest weight reduction. For the 2× and 3× rates of imazamox, all accessions responded similarly with the exception of Samos, the most widely-grown Greek cultivar, which had the highest values.

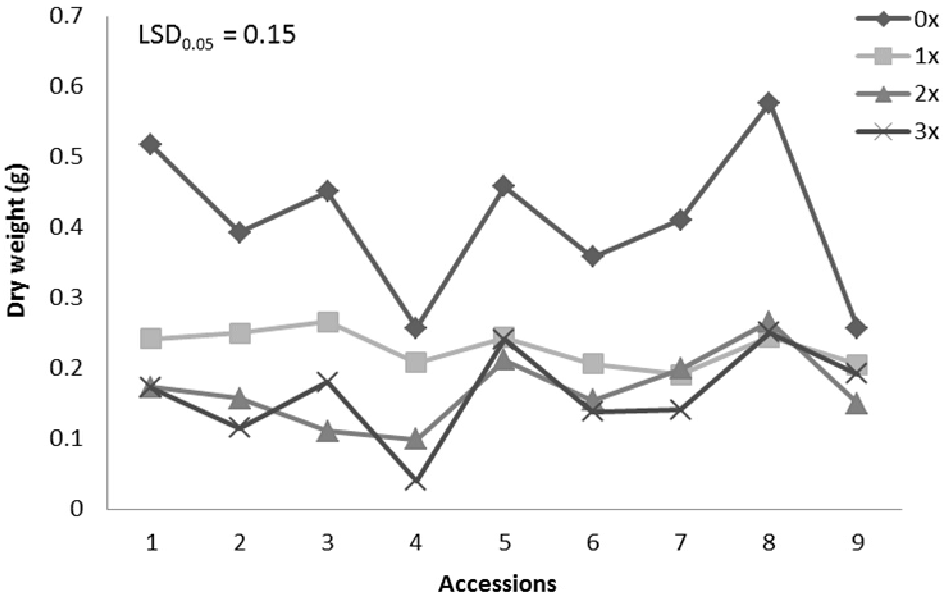

Figure 2. Effect of four rates of imazamox on the dry weight per plant of the nine lentil accessions tested in pots. Where 1= Dimitra, 2= Flip 2003-24L, 3= ILL-590, 4= ILL-6811, 5= Ikaria, 6= 73, 7= 81S 15, 8= Thessaly, 9= Samos.

The estimated GR_{50} values and the calculated R/S values [GR50 (R)/GR50 (S)] of the pot experiment indicated that cultivar Samos had the highest R/S value (12.9) while Dimitra was the most sensitive genotype to imazamox (Table 2). There was discordance between the R/S values of the *in vitro* test and the pot experiment suggesting no matching between the two methods. Similar findings have been previously reported by Simpson and Stoller (1996) who studied the effect of thifensulfuron and imazethapyr in sulfonylurea-tolerant soybean in laboratory (*in vitro*) and greenhouse experiments (*in vivo*); they concluded that the direct correlation between the results of *in vivo* and *in vitro* experiments was almost impossible. Such findings occur because herbicide concentration reaching the active center cannot be determined and its existed difference between *in vivo* and *in vitro* experiments differentiates its activity.

Table 2. Estimated GR_{50} using the above ground dry weights and the resistance ratios (R/S) of the nine lentil accessions tested in pots.

Accession	GR_{50}	R/S
Dimitra (S)	3.9	1.0
Flip 2003-24L	5.3	1.6
ILL-590	50.0	1.4
ILL-6811	6.7	1.7
Ikaria	6.3	3.0
73	6.3	1.6
81S 15	4.6	1.2
Thessaly	5.3	1.4
Samos	11.7	12.9

The previous discordance persisted and exacerbated in the field trial where all herbicide applications led to significant crop injury. The symptoms, consisting of foliar chlorosis, partial leaf burn, as well as stunting, are associated with ALS-inhibiting herbicides and have been reported in lentil and chickpea caused by imazephapyr (Erman et al., 2004; Taran et al., 2013). Although phytotoxicity symptoms declined over time due to plant re-growth, reduced plant growth, delayed flowering and maturity resulted in high yield losses. This finding is in agreement with previous reports where post-emergence application of imazethapyr and imazamox on different chickpea cultivars caused analogous losses (Taran et al., 2013). Consequently, the DW of imazamox treatments was reduced 35.9-66.1% compared to the untreated control (Table 3). However, the most influenced trait was SY; imazamox rates of 20 and 30 g ai ha^{-1} caused 90-97% yield reduction, while the 40 g ai ha^{-1} rate ramped up the losses to 84-99% compared to the untreated control of each accession (Table 3). The yield losses due to weed competition were estimated from 20-84% in lentil (Chaudhary et al., 2011; Sarker and Kumar, 2011). As a result of the SY collapse by imazamox application, HI decreased drastically (Table 3).

Apart from the intimate effect on lentil yield, weeds affect the produce indirectly degrading seed quality (Dangwal et al., 2010). Imazamox application led to PC increases in all cultivars, a finding which is fictitious as it is elicited by the reductions in TSW caused by imazamox and ranged from 14 to 56% (Table 3). Although TSW was affected by imazamox rates, the classification of the accessions, in general, was sustained with Ikaria and Thessaly to belong in macrosperma, while Dimitra, Flip 2003-24L and Samos to be microsperma. The reductions in TSW and the respective increases in PC were ascribed to the incomplete seed filling due to the delayed flowering and maturity under unfavorable conditions (high temperatures and low soil moisture).

Concluding, the results of the present study showed clearly that the Greek lentil accessions lack genes with resistance to imazamox, which makes necessary to investigate a broader genetic base or induce mutations in the already existing genetic material and subsequently to evaluate for possible modification of the ALS or AHAS enzyme.

Table 3. Mean comparisons of above-ground dry weight (DW), 1000-seed weight (TSW), seed yield (SY), harvest index (HI) and seed protein concentration (PC) in the five lentil accessions tested in the field.

Rate (g ai ha^{-1})	Accession	DW (kg ha^{-1})	TSW (g)	SY (kg ha^{-1})	HI (%)	PC (%)
0 (control)	Dimitra	4889.0	38.4	2062.3	38.2	30.7
	Flip2003-24L	5667.4	43.4	2675.4	42.9	28.8
	Ikaria	4469.6	75.6	1388.2	28.5	27.5
	Thessaly	5955.9	58.9	2597.5	39.8	27.6
	Samos	5526.6	45.7	2687.7	45.3	26.8
20	Dimitra	1749.2	29.0	136.0	6.8	31.0
	Flip2003-24L	1629.8	34.3	80.6	4.6	31.1
	Ikaria	2231.2	52.1	119.0	4.5	28.5
	Thessaly	2438.7	45.3	202.9	7.7	30.7
	Samos	2068.3	38.2	99.4	4.5	27.7
30	Dimitra	1748.5	29.7	97.3	4.6	31.1
	Flip2003-24L	1941.5	37.4	142.3	6.6	30.1
	Ikaria	2177.3	49.6	135.8	4.4	28.9
	Thessaly	2403.3	45.4	194.4	7.3	30.1
	Samos	1859.4	38.1	158.5	7.6	29.1
40	Dimitra	1462.3	27.1	93.9	5.7	30.0
	Flip2003-24L	1341.1	33.4	67.1	4.6	31.7
	Ikaria	1700.8	33.1	14.4	0.8	29.5
	Thessaly	2364.4	39.9	402.4	13.2	28.4
	Samos	1446.3	36.6	441.1	28.2	27.2
CV (%)		25.5	11.4	41.4	29.2	4.0
$LSD_{0.05}$		1182.9	7.9	476.1	7.5	2.0

Conclusions

Crop resistance to herbicides can be mainly due to alteration of the target site or metabolic detoxification of the herbicide. Developing one of these methods through genetic modification may provide herbicide resistance in a crop. However IMI-tolerant crops were achieved without insertion of foreign DNA and thus all commercial crops resistant to imidazolinones are non-transgenic but are available as non-GM plants under the trademark Clearfield®. Simple mechanisms conferring resistance to imidazolinones may be screened in the laboratory, whereas complex resistance mechanisms must be assessed in pots or/and under field conditions. Our study on Greek lentil accessions revealed no correlation between *in vitro* and *in vivo* experiments meaning that (1) the available material was not tolerant to imidazolinones and (2) different methods have to be used for assessing any potential tolerance.

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References

Al-Thahabi, S.A., Yasin, J.Z., Abu-Irmaileh, B.E., Haddad, N.I., Saxena, M.C., 1994. Effect of weed removal on productivity of chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Med.) in a Mediterranean environment. J. Agron. Crop Sci. 172, 333-341.

Ball, D.A., Ogg, A.G. Jr, Chevalier, P.M., 1997. The influence of seeding rate on weed control in small-red lentil (*Lens culinaris*). Weed Sci. 45, 296-300.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., Clayton, G.W., Thomas, A.G., Leeson, J.Y., Séguin-Swartz, G., Simard, M.J., 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86, 1243-1264.

Chaudhary, S.U., Iqbal, J., Hussain, M., Wajid, A., 2011. Economical weed control in lentils crop. J. Anim. Plant Sci. 21, 734-737.

Dangwal, R.L., Singh, A., Singh, T., Sharma, C., 2010. Effect of weeds on the yield of wheat crop in Tehsil Nowshera. J. Am. Sci. 6, 405-407.

Elkoca, E., Kantar, F., Zengin, H., 2005. Weed control in lentil (*Lens culinaris*) in eastern Turkey. New Zeal. J. Crop Hort. 33, 223-231.

Erman, M., Tepe, I., Yazlik, A., Levent, R., Ipek, K., 2004. Effect of weed control treatments on weeds, seed yield, yield components and nodulation in winter lentil. Weed Res. 44, 305-312.

Fedoruk, L.K., Shirtliffe, S.J., 2011. Herbicide choice and timing for weed control in imidazolinone-resistant lentil. Weed Technol. 25, 620-625.

Fedoruk, L.K., Johnson E.N., Shirtliffe, S.J., 2011. The critical period of weed control for lentil in Western Canada. Weed Sci. 59, 517-526.

Kaundun, S.S., Hutchings, S.J., Dale, R.P., Bailly G.C., Glanfield, P., 2010. Syngenta 'RISQ' test: a novel in-season method for detecting resistance to post-εmergence ACCase and ALS inhibitor herbicides in grass weeds. Weed Res. 51, 284-293.

Matus, A., Slinkard, A.E., Vandenberg, A., 1993. The potential of zero tannin lentil. In: Janick, J., Simon, J.E. (Ed), New Crops. Wiley, New York, pp. 279-282.

McDonald, G.K., Hollaway, K.L., McMurray, L., 2007. Increasing plant density improves weed competition in lentil (*Lens culinaris*). Anim. Prod. Sci. 47, 48-56.

McVicar, R., Pearse, P., Brenzil, C., Hartley, S., Panchuk, K., Mooleki, P., Vandenberg, A., Banniza, S. 2006. Lentil in Saskatchewan. [Online] Available at http://www.agriculture.gov.sk.ca/Default.aspx?DN=c5993bcc-009f-4031-b936-c52c992b9e7d (verified 18 December 2008). Saskatchewan Ministry of Agriculture.

Rainbolt, C.R., Thill, D.C., Zemetra, R.S., Shaner, D.L., 2009. Imidazolinone-resistant wheat acetolactate synthase in vivo response to imazamox. Weed Technol. 19, 539-548.

Rasheed, M., Jilani, G., Shah, I.A., Najeeb, U., Iqbal, T., 2010. Improved lentil production by utilizing genetic variability in response to phosphorus fertilization. Acta Agr. Scand. B-S P. 60, 485-493.

Sarker, A., Erskine, W., 2006. Recent progress in the ancient lentil. J. Agr. Sci. 144, 19-29.

Sarker, A., Kumar, S., 2011. Lentils in production and food systems in West Asia and Africa. Grain Legumes, 57, 46-48.

Seefeldt, S.S., Jensen, J.E., Fuerst, E.P., 1995. Log-logistic analysis of herbicide dose response relationships. Weed Technol. 9, 218-227.

Simpson, D.M., Stoller, E.W., 1996. Thifensulfuron and imazethapyr interaction at the ALS enzyme in sulfonylurea-tolerant soybean (*Glycine max*). Weed Sci. 44, 763-768.

Slinkard, A.E., Vanderberg, A., Holm, F.A., 2007. Lentil plants having increased resistance to imidazolinone herbicides. US Patent 7232942.

Tan, S., Evans, R.R., Dahmer, M.L., Singh, B.K., Shaner, D.L., 2005. Imidazolinone-tolerant crops: history, current status and future. Pest Manage. Sci. 61, 246-257.

Taran, B., Holm, F., Banniza, S., 2013. Response of chickpea cultivars to pre- and post-emergence herbicide applications. Can. J. Plant Sci. 93, 279-286.

Van Acker, R.C., Swanton, C.J., Weise, S.F., 1993. The critical period of weed control in soybean [*Glycine max* (L.) Merr.]. Weed Sci. 41, 194-200.

Vasilakoglou, I., Vlachostergios, D., Dhima, K., Lithourgidis, A., 2013. Response of vetch, lentil, chickpea and red pea to pre-or post-emergence applied herbicides. Span. J. Agric. Res. 11, 1101-1111.

Vencill, W.K., Nichols, R.L., Webster, T.M., Soteres, J.K., Mallory-Smith, C., Burgos, N.R., Johnson, W.G., McClelland, M.R., 2012. Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci. 60, 2-30.

Wall, D.A., 1996. Lentil (*Lens culinaris*) and fababean (*Vicia faba*) tolerance to post-emergence applications of imazethapyr. Can. J. Plant Sci. 76, 525-52.

Wall, D.A., McMullan, P.M., 1994. Effectiveness of several new selective herbicides in lentils (*Lens culinaris*). Crop Prot. 13, 553-557.

Yasin, J.Z., Al-Thahabi, S., Abu-Irmaileh, B.E., Saxena, M.C., Haddad, N.I., 1995. Chemical weed-control in chickpea and lentil. Int. J. Pest Manage. 41, 60-65.

Yenish, J.P., Brand, J., Pala, M., Haddad, A., 2009. Weed management. In: Erskine, W., Muehlbauer, F.J., Sarker, A., Sharma, B. (Ed.), the Lentil: Botany, Production and Uses. CAB International, Wallingford, pp. 326-342.