Genetic Variation for Tolerance to Herbicide Imazethapyr in Lentil (*Lens culinaris* Medik.)

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Identification of lentil genotypes tolerant to post-emergence herbicides and incorporation of herbicide tolerance in to high yielding varieties can prove a boon for developing effective integrated weed management strategy in lentil cultivation. A set of 180 diverse lentil genotypes was screened during two seasons for tolerance to post-emergence herbicide, imazethapyr. The herbicide imazethapyr was sprayed at 75 g a.i. ha^{-1} at 50 days after sowing and genotypes were scored initially after 14 days of spray and thereafter 45 days of spray when plants showed recovery. Large variations among the genotypes were observed for tolerance to imazethapyr herbicide. On a 1-5 scale, 12 genotypes were found tolerant, 46 moderately tolerant, 112 sensitive and 10 highly sensitive during the first season, while in the second season, 11 genotypes were found tolerant, 51 moderately tolerant, 110 sensitive and 8 highly sensitive. Based on screening during the first season, a small set of 30 genotypes, including some tolerant, moderately tolerant, sensitive and highly sensitive, was evaluated to determine the effect of herbicide on morpho-physiological traits, yield and yield components. Adverse effect of imazethapyr herbicide was observed for plant height,

days to 50% flowering and pod initiation, biomass accumulation, leaf area index, specific leaf weight, crop growth rate, chlorophyll content, 100-seed weight, pods plant^{-1} and seed yield when compared to control (unsprayed). Tolerant genotypes showed less reduction for most of the morpho-physiological traits, yield components and yield. Five genotypes namely LL699, LL1397, IPL406, EC78452 and LL1203 demonstrated higher herbicide tolerance with less adverse effect on various morpho-physiological traits and recorded less reduction (<19%) for seed yield in imazethapyr treated plots as compared to control. These genotypes can be used in breeding programme for developing post-emergence herbicide tolerant cultivars and for genetic studies. The development of improved herbicide tolerance is critical to improving weed control in lentil, and expanding lentil production into new areas to support the practice conservation agriculture.

Nomenclature: Imazethapyr; lentil, *Lens culinaris* Medik.

Keywords: post-emergence herbicide, morpho-physiological traits, chlorophyll content

Lentil (*Lens culinaris* Medik.) is one of the important grain legumes cultivated extensively in India, Canada, Turkey, Bangladesh, Iran, China, Nepal and Syria. In India, it is the second most important cool season food legume crop next to chickpea and is grown on about 1.42 million hectares with a production of 1.13 million tonnes (Agropedia, 2012). It has the ability to thrive well on relatively poor soils and adverse environmental conditions and is relatively tolerant to drought and harsh winters. Yield of lentils generally poor due to management problems and susceptibility to various biotic and abiotic stresses. Weeds continue to have a major impact on lentil production. Being a poor weed competitor, most annual grass and broadleaf weed species can compete effectively with lentil throughout the growing season for nutrients, water, and light, reducing crop yields and grain quality and also harbour insect-pests and diseases. Yield losses due to weed

competition vary considerably depending on the level of weed infestation and weed species prevailing. Yield losses in lentil of 20-80%, as a result of weeds (Saxena and Wassimi 1980; Chaudhary and Singh 1987; Al-Thahabi et al. 1994; Halila 1995; Yenish et al. 2009) and the critical period lies between 30-60 days after sowing (Yaduraju and Mishra 2005). Therefore, weed management in early stage is crucial to realize maximum yield as well as to maintain good quality of produce in lentil.

In lentil, weeds are generally controlled manually. However, hand weeding, which is labour-intensive and an expensive operation, is impractical in the extensive production areas (Bhan and Kukula 1987; Saxena 1990) and if delayed, the operation does not prevent the adverse effect of the weeds on crop yield (Mohamed et al. 1997). The use of appropriate herbicides can eliminate the crop-weed competition and prevent yield losses in lentil production (Muehlbauer et al. 1995). It is therefore necessary that effective herbicides are used to reduce unwanted competition. The pre-emergence herbicides are effective in controlling weeds at early stage of seedling growth, but weeds germinating after crop emergence become dominant in the field and cause substantial yield losses. The choices for post-emergence herbicides are limited because of the sensitivity of lentil to broad-spectrum herbicides like amino acid synthesis inhibitor (acetolactate synthase enzyme) family imidazolinones (IMI) and sulfonylureas, lipid inhibitor (acetyl-CoA carboxylase enzyme) family cyclohexanediones and the growth regulator inhibitor family phenoxyacetic acid and benzoic acid. Therefore, lentil cultivars with improved herbicide tolerance, which can offer greater flexibility for use of broad spectrum post-emergence herbicides, are required by the farmers.

The IMI class of herbicides, including imazethapyr, imazamox and imazapic provides a broad spectrum of weed control activity, flexibility in timing of application, low usage rates, and low mammalian toxicity (Tan et al. 2005). IMI-tolerant genotypes have been identified in many

species, which has enabled the development of several tolerant crops (Miller and Al-Khatib 2002; Tan et al. 2005).

Herbicide tolerant cultivars have been developed in many crops, including some grain legumes, by exploiting available genetic variability in the germplasm or by inducing variability through mutagenesis. For example, a variety 'Coromup' in narrow leaf lupin (*Lupinus angustifolius* L.) (Si et al. 2008) and a variety 'Tracy-M' in soybean (Hartwig 1987) with improved tolerance to post-emergence herbicide metribuzin were developed by screening the advanced breeding lines. In narrow leaf lupin, two highly tolerant metribuzin mutants, Tanjil-AZ-55 and Tanjil-AZ-33, were identified (Si et al. 2009). There are a few reports on identification of herbicide tolerance in chickpea (Taran et al. 2010; Si et al. 2010). Recently it has been reported that large genetic variability exists in chickpea for herbicide tolerance (Gaur et al. 2013; Chaturvedi et al. 2014). Similarly in lentils, a variety 'RH44' was developed by mutagenesis and cross-breeding that has an increased tolerance to IMI herbicide (Slinkard et al. 2007). They further suggested that there are genotypic variations for herbicide tolerance in lentil which could be utilized for development of herbicide tolerant cultivars. Therefore, lentil cultivars with improved herbicide tolerance are very much needed to enhance options for weed control by post-emergence herbicides which provide weed control for longer period and results in increased productivity. The present investigation therefore was undertaken to evaluate the variation of diverse lentil germplasm for tolerance to post-emergence herbicide, imazethapyr and to identify sources of tolerance for potential use in development of herbicide tolerant cultivars.

Materials and Methods

Experimental site. The soil of the experimental site was loamy sand in texture (Typic Ustochrepts). The top 0-15 cm layer of the soil profile was neutral in pH (7.3), with 0.11 dS m^{-1}

electrical conductivity, low in $KMnO_4$-oxidizable N (177.3 kg ha^{-1}) and Walkley and Black organic carbon (0.28%), medium in Olsen- P (15.6 kg ha^{-1}) and 1 N NH_4OAc-extractable K (149.8 kg ha^{-1}). The meteorological data recorded at meteorological observatory of Punjab Agricultural University, Ludhiana indicated that rainfall received during the crop season was 230.0 and 213.0 mm in 2013-14 and 2014-15, respectively. The crop experienced mean weekly maximum temperatures ranging from 15.1 to 28.8ºC and 12.5 to 32.6ºC during 2013-14 and 2014-15, respectively, whereas the mean weekly minimum temperature ranged from 3.8 to 15.1ºC and 5.2 to 18.1ºC during 2013-14 and 2014-15, respectively.

Plant materials, experimental design and details. The present study includes two experiments which were conducted at Punjab Agricultural University, Ludhiana, India during *rabi* (winter) seasons of 2013-14 and 2014-15. In first experiment, 180 diverse lentil genotypes were screened for herbicide tolerance against imazethapyr. These genotypes were acquired from different national and international institutes including NBPGR, New Delhi, India and ICARDA, Rabat, Morocco. Sowing was done on November 19, 2013 and November 14, 2014. The experiment was conducted in two different sets in Alpha Lattice Design. These sets include Set-1 (imazethapyr treated plots) and Set-2 (unsprayed control plots). Each genotype was accommodated in a plot of 2 rows of 1 m length keeping row to row spacing of 22.5 cm. The herbicide imazethapyr was sprayed @ 75 g a.i. ha^{-1} at 50 days after sowing with a shoulder-mounted hand operated knapsack sprayer using 375 L ha^{-1} of water. For uniform spread and absorption, imazethapyr was mixed with cyboost (700 g ha^{-1}) and cyspread (565 ml ha^{-1}) in the spray solution. The spraying was done during cooler hours of the day when there was little or no wind. In second experiment, a small set of 30 genotypes including some tolerant, moderately tolerant, sensitive and highly sensitive genotypes, was selected based on screening of first season (2013-14). These 30 genotypes were grown in two

sets (herbicide treated and control plots) to determine the effect of herbicide on various morpho-physiological traits, yield components and yield. Each genotype was accommodated in a plot of 4 rows x 4 m length in Factorial Randomized Complete Block Design with three replications.

Scoring of genotypes. The genotypes were scored at 14 and 45 days after herbicide spray on a 1-5 scale for herbicide tolerance as proposed in chickpea by Gaur *et al*. (2013), where, 1=Highly tolerant (excellent plant appearance, no chlorosis/narrowing/burning of leaves); 2=Tolerant (good plant appearance with minor chlorosis/narrowing/burning of leaves); 3=Moderately tolerant (fair plant appearance with moderate chlorosis/narrowing/burning of leaves); 4=Sensitive (poor plant appearance with severe chlorosis/narrowing/burning of leaves); and 5=Highly sensitive (complete burning of leaves leading to mortality of most of the plants). The average scores over the replications were used to classify genotypes in different categories.

Recording of observations on morpho-physiological traits, yield components and yield. In second experiment, various morpho-physiological traits, yield components and yield were recorded on 30 genotypes in imazethapyr treated and control plots to determine the effect of herbicide spray.

Days to 50% flowering and days to pod initiation were recorded as the number of days taken from the date of sowing to 50% flowering and pod initiation on plot basis. Five random plants were taken to measure plant height (cm) from the base of plant to the tip of the main shoot at the time of maturity using meter rod. The leaf are index (LAI) was recorded using sun scan canopy analyser instrument at 115 days after sowing when crop attained maximum growth.

The specific leaf weight (SLW) was recorded by taking leaf area at 115 days after sowing and then the leaves were subsequently dried for 48 hrs at 60°C. The dried leaf samples were used to measure SLW by dividing dry weight of leaves to leaf area. The crop growth rate (CGR) expressed

in grams per square meter per day (g m^{-2} d^{-1}) was calculated at 75 and 90 days after sowing using the following formula (Watson, 1952).

$$CGR = (W_2-W_1)/ (t_2-t_1) \times (1/SA);$$

Where, W_1 and W_2 = crop dry weight at the beginning and at the end of the 15 days interval, t_1 and t_2 = corresponding days, SA = soil area occupied by the plants at each sampling.

The chlorophyll content, an indirect measure of photosynthetic efficiency, was estimated at 105 days after sowing for 8 genotypes including 4 tolerant and 4 sensitive or highly sensitive genotypes. The middle leaves of 5 random plants from each genotype were taken to determine chlorophyll content using method given by Anderson and Boardman (1964). The optical density (OD) of the chlorophyll fraction was measured at 645 and 663 nm against the 80% acetone as a blank on the spectrochem spectrophotometer.

The biomass of the five randomly selected plants were recorded at pod initiation stage and oven dried at 80^0C till uniform constant weight was obtained. The average of five plants was taken as biomass $plant^{-1}$ and expressed in grams. The number of pods present in five randomly selected plants at maturity was counted individually and the average was worked out and expressed as pods $plant^{-1}$. The 100-seed weight was recorded in each genotype by adopting ISTA procedure (Anonymous, 1999) and was expressed in grams. The yield $plot^{-1}$ was recorded by harvesting two middle rows of each genotype and threshed and weighed in grams and was converted in to kg ha^{-1}.

Recording of observations on type and number of weeds. Data were also recorded on types and number of weeds both before and after the herbicide spray. No weeding was done in herbicide treated plots, while the control plots were kept weed free with two hand-weeding. The number of different weeds was counted in a 1.0 m^2 area from 10 spots. The effect of imazethapyr herbicide on

different weeds was compared with two hand-weeding to determine the efficacy of herbicide on different weeds.

Statistical analysis. The data collected on various parameters under study were subjected to statistical analysis as per the procedure given by Snedecor and Cochran (1989) and adapted by Cheema and Singh (1991). All comparisons were made at 5% level of significance.

Results and Discussion

Herbicide tolerance. Visual symptoms started appearing on plants about 10 days after imazethapyr spray. Imazethapyr killed the growing tips (epical meristem and young leaves) of the branches and affected the vegetative growth of the sensitive genotypes. A high level of injuries on various plant parts was observed in some highly sensitive genotypes which led to 70% to 85% mortality of plants. Effect of imazethapyr on different lentil genotypes was ranged from very little to very high injuries including chlorosis or burning of leaves (Fig. 1). Other abnormalities like stunted growth, very small or needle shaped leaves, leaf narrowing, reduction in plant biomass, reduction in crop growth, delayed flowering, delayed pod initiation, poor pod setting and reduction in pod number and 100-seed weight were also observed.

Different ratings scales have been proposed by various workers for herbicide tolerance. Based on plant injury, 0–9 scale was reported to be rapid and reliable measurement of tolerance to herbicides in chickpea (Taran et al., 2010), but this scale is tedious and based on plant injuries only, whereas chickpea and lentil plants also show chlorosis and narrowing of leaves, besides plant injury or burning of leaves after herbicide spray. Therefore, a more simplified 1-5 scale as proposed by Gaur et al. (2013) for chickpea was used in the present study. To our knowledge, this is the first study of screening of lentil germplasm for tolerance to imazethapyr. The severity of symptoms

varied with lentil genotypes from no visible injury to severe chlorosis and complete death of plants of highly sensitive genotypes.

Accessions were grouped based on the herbicide injury scale (1-5) at 14 and 45 days after spray. The mean herbicide tolerance score ranged from 1.5 to 5.0 after 14 days of spray. After 30 days of spray, most of the genotypes showed some symptoms of recovery during both the seasons, thus they were scored again for herbicide tolerance after 45 days of spray and the score ranged from 1.5 to 4.5. The genotypes were grouped into different categories based on herbicide tolerance score during both seasons (Table 1). None of the genotypes was found highly tolerant in either season, however a large number of genotypes were found sensitive and moderately tolerant, besides some tolerant and highly sensitive genotypes. In both the seasons, large natural genetic variability was observed against the post-emergence application of imazethapyr herbicide (Fig. 2). Out of 180 tested genotypes, 12 genotypes were found to be tolerant, 46 moderately tolerant, 112 sensitive and 10 highly sensitive to imazethapyr during *rabi* 2013-14. Similarly in second season (2014-15), 11 genotypes were found to be tolerant, 51 moderately tolerant, 110 sensitive and 8 highly sensitive. Most of the genotypes were found consistent in their reaction to imazethapyr, however four genotypes, which were sensitive in first season, changed their category to moderately tolerant and two genotypes, which were found highly sensitive in first season, changed their category to sensitive in second season. The change in category of a few genotypes from highly sensitive to sensitive or from sensitive to moderately tolerant in second season seems to be due to micro-environment in that vicinity which might have reduced the efficacy of herbicide. Majority of the genotypes showed regeneration in imazethapyr treated plots after about 30 days of spray, indicated that adverse effect of herbicide was reduced and plants showed recovery. However for final grouping, the highest score recorded by a genotype in either season was taken into consideration.

Overall, 11 genotypes were found tolerant, 47 as moderately tolerant, 112 as sensitive and 10 as highly sensitive. Average herbicide tolerance score of some tolerant, moderately tolerant, sensitive and highly sensitive genotypes are given in Table 2.

The IMI class of herbicides is very effective for controlling a broad range weed in cereal-based cropping systems and in a number of legume crops. Some legumes and many oilseed crops are sensitive to IMI herbicides and could be severely injured by the presence of herbicide in the soil at a level as low as 0.5 parts per billion, or less than 5% of the recommended rates for weed control (Stork 1995). Grouping of genotypes in different categories revealed large genetic variability in the germplasm for tolerance to imazethapyr herbicide as also reported earlier in lentil (Slinkard et al. 2007), chickpea (Gaur et al. 2013; Chaturvedi et al. 2014) and ryegrass (Preston and Powles 2002). Another study showed the existence of wide range of natural variation for tolerance to IMI group of herbicides (imazethapyr and imazamox) in chickpea (Taran et al. 2010) and fieldpea (Hanson and Thill 2001). The IMI group of herbicides is registered in Turkey for the control of broadleaf weeds in chickpea (Kantar et al., 1999). Efforts have also been made to induce resistance to IMI class of herbicides in chickpea by induced mutagenesis through gamma rays (Toker et al., 2012). The results indicated the possibilities to isolate highly tolerant genotypes through screening of large number of germplasm lines to exploit natural variation or through induced mutations.

In case of second experiment, the herbicide tolerance score of 30 genotypes ranged between 1.5 and 4.25, and of these, 5 genotypes were found tolerant, 18 moderately tolerant, 5 sensitive and 2 highly sensitive to imazethapyr herbicide. It was observed that all these genotypes were grouped in similar category in previous season. Among the tolerant genotypes, LL1203 and LL1397 had the lowest score (1.5) followed by IPL406, LL699 (1.83) and EC78452 (2.0), while among the highly sensitive genotypes, IPL327 had the highest score (4.25) followed by LL1365

(4.16). In case of imazethapyr treated plots, majority of the genotypes showed some symptoms of recovery after about 30 days of spray, except four genotypes EC28514, LL1365, LL1374 and LL931 which did not show any recovery.

Effect of imazethapyr spray on morpho-physiological traits. The analysis of variance revealed significant differences between the treatments (herbicide v/s control plots) and among the genotypes for all the morpho-physiological traits in case of treated and control plots (data not shown). Data of herbicide treated and control plots and per cent reduction for various traits of some selected tolerant, moderately tolerant, sensitive and highly sensitive genotypes are given in Tables 3, 4, and 5.

Days to 50% flowering and days to pod initiation. Days to 50% flowering in control ranged from 95 to 109 days with a mean value of 98.2 days, while in imazethapyr treated plots it ranged from 100 to 118 days (Table 3) with a mean value of 105.6 days, thus recorded an overall delay of 7 days. Six genotypes namely EC78436, EC78452, EC255491, EC267558, EC267544 and IPL406 showed minimum delay (2 days), while LL1361 was having maximum delay (20 days) followed by EC267564 and EC267537 (17 days) for days to 50% flowering (Table 3).

Days to pod initiation in control plots ranged from 102 to 114 days with a mean value of 105.4 days, while in imazethapyr treated plots, it ranged from 108 to 129 days with a mean value of 115.4 days, thus recorded an overall delay of 10 days. Two genotypes, EC78455 and EC223294, showed the minimum delay (2 days) followed by EC267677 (3 days), while the genotype EC267662 showed maximum delay (20 days) followed by IPL326 and EC267572 (19 days) for days to pod initiation (Table 3). In general, it was observed that the tolerant genotypes were having less delay for days to 50% flowering and pod initiation than the sensitive genotypes.

The significant differences between the herbicide treated and control plots for days to 50% flowering and pod initiation revealed that herbicide spray adversely affected these two traits and delayed the flowering and pod initiation. Delay in days to 50% flowering seems to be due to temporary arrest of the growth of plant after herbicide spray. Similar findings have been reported by Taran et al. (2013) in chickpea in which they found that pre-emergence application of imazethapyr or post-emergence application of metribuzin delayed flowering. Delay in pod initiation is obviously due to delay in days to 50% flowering and due to slow crop growth rate of herbicide treated plots, which ultimately resulted in delayed maturity. Findings of Royuela et al. (2000) suggests that growth of peas plants inhibited after spray of imazethapyr due to starvation and blockage of acetolactate synthase catalyzed reactions.

Plant height and biomass plant^{-1}. A considerable reduction of mean plant height (24.6%) was observed in the imazethapyr treated plots as compared to control plots. The reduction in the plant height was ranged from 43.5% in highly sensitive genotype LL1365 to 5.0% in tolerant genotype LL1397 (Table 3) in the imazethapyr treated plots. Overall it was found that reduction was less in tolerant genotypes and more in sensitive or highly sensitive genotypes. On the average of 30 genotypes, biomass plant^{-1} showed a reduction of 35.1% and the maximum reduction (62.2%) was observed in highly sensitive genotype LL1365, while the minimum reduction (11.1%) was recorded by tolerant genotype LL699 in the imazethapyr treated plots (Table 3). Imazethapyr inhibits the activity of acetolactate synthase (ALS enzyme), which is involved in the synthesis of the branched chain amino acids like leucine, isoleucine, and valine (Stidham 1991). ALS herbicides are easily absorbed by both roots and foliage and later on translocated in both the xylem and the phloem to the site of action, i e growing points (Peterson et al. 2001). In growing points, it inhibits the ALS enzyme, causing death of meristematic cells resulting in plant death (Little and Shaner 1991).

Reduction of plant height and biomass in herbicide treated plots was also reported earlier in chickpea by Taran et al. (2013). Impairment of ALS activity may lead to fermentative metabolism that causes overall growth inhibition in peas (Gaston et al. 2002). The varied level of tolerance in different genotypes may be attributed to differential metabolic degradation rate.

Leaf area index (LAI). Leaf area index, a measure of plant canopy development and contributes towards grain yield, was adversely affected by imazethapyr spray. The maximum reduction (61.2%) in LAI was observed in highly sensitive genotype LL1365, while the minimum reduction (10.1%) was recorded in tolerant genotype LL699 (Table 4). It was observed that LAI of all 30 genotypes was reduced significantly in herbicide treated plots, however the reduction was less in tolerant as compared to sensitive or highly sensitive genotypes. During visual scoring, leaf narrowing was observed as a peculiar character after imazethapyr spray which reduced the leaf area index, indicated that the leaf narrowing adversely affected the photosynthetic and amino acid activity of lentil genotypes which led to poor canopy coverage of the treated plots and ultimately reduced grain yield. Reduction in leaf area due to herbicide imazamox spray was also observed in strawberry which ultimately reduced the yield (Miller, 2003).

Chlorophyll content. Chlorophyll content, an indirect measure of photosynthetic efficiency, was estimated in selected 8 genotypes including 4 tolerant and 4 sensitive to highly sensitive genotypes. The quantity of total chlorophyll content (mg chl g^{-1} fresh tissue) ranged from 3.6284 to 4.4808 in control plots and from 2.0172 to 4.1363 in imazethapyr treated plots. Significant reduction in chlorophyll content in herbicide treated plots was observed for all the 8 genotypes as compared to control plots, however reduction in tolerant genotypes (EC78452, IPL406, LL1397, LL699) was <10.0%, while in case of sensitive or highly sensitive genotypes it ranged from 20.2 to 47.7%. Herbicide spray either damage the plant parts or kill the plant completely by disrupting

essential physiological and biochemical processes which cause ion leakage from cells and changes in energy allocation to the photo system. In present study, the chlorophyll content was reduced significantly which probably lead to the reduction in rate of leaf photosynthesis and ultimately reduction in yield.

Crop growth rate (CGR) and specific leaf weight (SLW). In case of imazethapyr treated plots, significant reduction in the overall mean CGR and individual genotypes was observed as compared to control plots. The overall reduction in CGR in herbicide treated plots was 32.1%. The minimum reduction in CGR (8.8%) was recorded in tolerant genotype LL1397 and the maximum reduction (63.8%) was recorded in highly sensitive genotype DPL15 followed by LL1377 (63.7%) (Table 4). The significant reduction in the overall mean CGR as well as for all the genotypes individually indicated that herbicide spray suppressed the growth of genotypes. However, the varied level of suppression for growth attributed to variation in tolerance level of different genotypes. Similarly, the overall mean SLW was reduced significantly in imazethapyr treated plots. It was also reduced significantly in 23 out of 30 genotypes. The minimum reduction (3.1%) was observed in tolerant genotype LL699, while the maximum reduction of 30.4% was observed in highly sensitive genotype LL1365 (Table 4).

Yield components. Pods plant^{-1} is the most important yield contributing component for which the minimum reduction (8.1%) was observed in tolerant genotype LL699, while the maximum reduction (52.1%) was observed in highly sensitive genotype LL1365 in imazethapyr treated plots (Table 5). In general, the tolerant genotypes showed less reduction, whereas sensitive or highly sensitive genotypes showed more reduction for pods plant^{-1}. The 100-seed weight is also an important yield contributing trait which was also adversely affected by imazethapyr spray and overall a reduction of 11.9% was recorded in herbicide treated plots as compared to control. The

minimum reduction (1.2%) was recorded in tolerant genotype IPL406, while the maximum reduction (23.0%) was recorded by highly sensitive genotype LL1365 (Table 5). In imazethapyr treated plots, significant reduction for pods plant^{-1} might be attributed to reduced crop growth rate, while the reduced 100-seed weight might be due to reduced reproductive phase resulting from delayed flowering and pod initiation. It also adversely affected the photosynthetic efficiency of the plants which lead to poor accumulation and translocation of photosynthates and finally poor source-sink relationship which lead to reduced seed size. Reduction in seed size has also been observed earlier in herbicide treated lentil plots (Frisen and Wall 1986).

Seed yield. Seed yield of lentil genotypes was influenced significantly due to Imazethapyr application in both the experiments. In case of first experiment, the overall mean of 180 genotypes for seed yield in control and imazethapyr treated plots was respectively 1107 kg ha^{-1} and 789 kg ha^{-1} during first season, while during second season, it was 1102 kg ha^{-1} and 807 kg ha^{-1}, respectively (Data not shown). The seed yield reduction ranged from 10.0% to 61.9%.

In second experiment, the overall mean of 30 genotypes for seed yield was 1155 kg ha^{-1} and 782 kg ha^{-1}, respectively in control and imazethapyr treated plots and recorded overall reduction of 32.3% in imazethapyr treated plots. The minimum reduction (9.6%) for seed yield was observed in tolerant genotype LL699, while the maximum reduction (67.2%) was recorded by highly sensitive genotype LL1365 (Table 5). The herbicide spray caused reduction for most of the traits such as plant height, crop growth rate, chlorophyll content, pods plant^{-1} and 100-seed weight ultimately reduced seed yield of lentil genotypes. In general, the yield reduction was less in tolerant genotypes as compared to sensitive or highly sensitive genotypes. Frisen and Wall (1986) also found tolerance in lentil against metribuzin herbicide and varied level of yield reduction corresponding to tolerance level. Considerable yield reduction was also reported earlier in chickpea which was corresponds to

the severity of visual injury symptoms (Taran et al. 2013). The reduction for most of the traits including seed yield was directly proportional to the level of tolerance or sensitivity of genotypes in the present study. This further supports that the visual scoring on 1-5 scale was efficient for grouping of genotypes for herbicide tolerance in to various categories.

However, contrary to results of present study, Hanson and Thill (2001) did not find significant yield reduction in lentil and pea after spray of imazethapyr and pendimethalin combination. The reason for non-significant yield reduction was attributed to low dose of imazethapyr and pre-emergence application of pendimethalin which is less toxic as compared to post-emergence application.

Weed flora and effectiveness of imazethapyr for their control. Weed species killed by imazethapyr indicated that the weed control was effective by the tested herbicide. These results suggest that the post-emergence application of imazethapyr herbicides by developing herbicide tolerant cultivars will be an effective strategy for weed management in lentil fields. Imazethapyr, an IMI compound, is used as selective herbicide to control most annual grasses and certain broad leaf weeds. Imazethapyr inhibits amino acid synthesis and can be applied as pre-emergence as well as post-emergence herbicide in legume fields (Manijeh and Jagannath 2011). They provide flexibility in application time, lower rates of application and have low mammalian toxicity (Tan et al. 2005). These compounds act by inhibiting acetolactate synthase (ALS, E.C. 4.1.3.18), a key enzyme in the synthesis of branched-chain amino acids like valine, leucine and isoleucine (Stidham 1991). After being translocated through phloem, it inhibits ALS resulting in the death of meristematic cells and finally the whole plant (Little and Shaner 1991).

As far as weed flora was concerned, a number of monocot and dicot weeds were observed in the field. The major weed species in the experimental field included *Oenothera drumundii* (Railway

creeper), *Cyperus rotundus* (Purple nut sedge), *Medicago denticulata* (Toothed bur clover) and *Lepidium sativum* (Pepper grass) (Table 6). The effect of herbicide spray on weeds was compared with successive two hand-weeding (30 and 60 DAS). Most of the weeds were controlled by imazethapyr. Though the population of *Oenothera drumundii*, *Cyperus rotundus*, *Medicago denticulata* and *Lepidium sativum* and their dry weight was significantly higher with post-emergence application of imazethapyr at 75 g a.i. ha^{-1} over two hand weeding at 30 and 60 DAS, yet it was able to control the prominent weeds effectively. These results confirm that variation in tolerance to imazethapyr exists within lentil germplasm. The existence of tolerance to IMI herbicides is seen in maize, wheat, rice, oilseed rape, sunflower (Siyuan et al. 2005) and field pea (Hanson and Thill 2001). ALS inhibiting herbicides mode of inheritance is relatively simple with a single, dominant nuclear gene in *Xanthium strumarium* (Lee and Owen 2000) and *Galium spurium* (Van Eerd et al. 2004), or a single, partially dominant gene in *Sonchus oleraceus* (Boutsalis and Powles 1995) and sunflower (Kolkman et al. 2004).

Genetical studies of herbicide (IMI) tolerance in many crops indicated that tolerance is governed by single dominant gene (Chant 2004). The genetics of the herbicide tolerance against different groups of herbicides has been studied in some legume crops such as soybean and lupin. Edwards et al. (1976) reported that a single recessive gene was responsible for controlling metribuzin sensitivity. Similarly, Karazawa and Caviness (1979) identified a few genes exhibiting partial dominance which were responsible for resistance to propanil injury, whereas Glover and Schapaugh (2002) observed a complex pattern of inheritance for pendimethalin injury in soybeans. Another study revealed a single dominant gene in two soybean cultivars for tolerance to sulfentrazone herbicide (Swantek et al. 1998). In *Medicago* species, a single dominant gene was found to control tolerance for sulfonylurea (SU) herbicide (Oldach et al. 2008), while in narrow-leaf lupin, a single partially

dominant gene was reported for resistance to metribuzin herbicide (Si et al. 2011). In lentil also, genetic studies can be performed by developing appropriate populations by using the tolerant and sensitive sources against imazethapyr. It will help to incorporate herbicide tolerance in high yielding lentil cultivars.

Herbicide tolerant lentil cultivars are very much needed to enhance options for application of post-emergence herbicides. Weed management through herbicides is needed even in the developing countries, such as India, to make lentil cultivation more profitable. Weed management through herbicides is not only economical but also facilitates zero-tillage or minimum tillage methods, which help in practicing conservation agriculture. The screening of diverse set of lentil germplasm provided a good indication of the presence of natural genetic variability for imazethapyr tolerance and it would encourage further screening of large germplasm collection to search for even more robust and diverse sources for herbicide tolerance. The herbicide tolerant genotypes identified in present study would be useful in physiological and genetic studies and in the development of herbicide tolerant cultivars. The availability of the sources of herbicide tolerance and vast genomics resources would help in hasty progress in the development of herbicide tolerant lentil cultivars in near future.

Acknowledgements

This work was undertaken as part of, and funded by, the CGIAR Research Program on Grain Legumes in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA).

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Table 1. Grouping of lentil genotypes based on Imazethapyr herbicide tolerance

Category	Number of genotypes		Final grouping of genotypes
	2013-14	2014-15	
Highly tolerant	0	0	0
Tolerant	12	11	11
Moderately tolerant	46	51	47
Sensitive	112	110	112
Highly sensitive	10	8	10
Total genotypes	**180**	**180**	**180**

Table 2. Imazethapyr herbicide tolerance scores of some tolerant, moderately tolerant, sensitive and highly sensitive lentil genotypes

Genotype	2013-14	2014-15	Average ± SE	Category
IPL406	1.50	1.83	1.67 ± 0.17	Tolerant
LL699	2.00	1.83	1.92 ± 0.09	Tolerant
EC78452	2.00	2.00	2.00 ± 0.01	Tolerant
LL1203	2.00	1.50	1.75 ± 0.25	Tolerant
LL1397	2.00	1.50	1.75 ± 0.25	Tolerant
LL1369	2.50	2.67	2.58 ± 0.09	Moderately Tolerant
LL1385	2.17	2.25	2.21 ± 0.04	Moderately Tolerant
PL406	4.00	3.33	3.67 ± 0.36	Sensitive
LL931	3.50	3.00	3.25 ± 0.25	Sensitive
LL1365	4.50	4.16	4.33 ± 0.17	Highly Sensitive
IPL327	4.00	4.25	4.13 ± 0.13	Highly Sensitive

Table 3. Means value of some tolerant, moderately tolerant, sensitive and highly sensitive genotypes for days to 50% flowering, pod initiation, plant height and biomass plant^{-1} in treated and control plots

Genotypes	Days to 50% flowering			Days to pod initiation			Plant height (cm)			Biomass plant^{-1} (g)		
Tolerant genotypes												
Genotypes	**ITP**	**CP**	**Delay**	**ITP**	**CP**	**Delay**	**ITP**	**CP**	**% Reduction**	**ITP**	**CP**	**% Reduction**
EC78452	101	99	2	110	106	4	26.7	28.5	6.4	4.20	5.23	19.8
IPL406	100	98	2	115	104	4	26.5	28.1	5.8	3.32	4.74	30.1
LL699	103	99	4	118	103	6	28.7	30.3	5.5	4.90	5.52	11.1
LL1203	103	100	3	118	107	5	27.6	29.2	5.5	4.58	5.63	18.7
LL1397	103	99	4	112	103	5	27.5	28.9	5.0	5.12	6.23	17.8
Moderately tolerant genotypes												
LL1369	101	97	4	117	104	9	18.3	22.8	32.7	2.74	4.74	42.7
LL1385	102	97	5	114	105	8	19.4	28.2	31.0	3.41	5.06	32.5
Sensitive genotypes												
PL406	106	98	8	114	106	8	17.9	25.5	29.9	2.45	3.61	32.3
LL931	109	97	9	121	104	10	23.2	29.5	21.2	3.16	5.13	38.4
Highly sensitive genotypes												
IPL327	108	97	11	116	106	12	14.7	24.9	41.2	1.22	3.14	61.0
LL1365	108	100	10	115	106	11	11.7	20.6	43.5	1.25	3.31	62.2
Overall mean*	**105.6**	**98.2**	**7.4**	**115.4**	**105.4**	**10.1**	**20.5**	**27.2**	**24.6**	**2.8**	**4.32**	**35.1**
CD (5%)	**Treatments (T)**		**1.5**			**1.8**			**0.6**			**0.06**
	Genotypes (G)		**5.3**			**6.4**			**2.3**			**0.21**
	Interaction (T x G)		**8.4**			**9.2**			**3.2**			**0.30**

*: Mean of 30 genotypes; ITP: Imazethapyr treated plots, CP: control plots; NS: non-significant

Table 4. Means value of some tolerant, moderately tolerant, sensitive and highly sensitive genotypes for leaf area index, specific leaf weight and crop growth rate in treated and control plots

Genotypes	Leaf Area Index			Specific leaf weight ($g\ cm^{-2}$)			Crop growth rate ($g\ m^{-2}\ d^{-1}$)		
Tolerant genotypes									
Genotypes	**ITP**	**CP**	**% Reduction**	**ITP**	**CP**	**% Reduction**	**ITP**	**CP**	**% Reduction**
EC78452	2.47	2.97	16.85	0.00586	0.00617	5.0	7.47	9.00	17.0
IPL406	2.33	2.80	16.67	0.00539	0.00567	5.0	6.61	7.59	29.2
LL699	3.57	3.97	10.08	0.00484	0.00500	3.1	8.00	8.94	10.6
LL1203	2.20	2.63	16.58	0.00595	0.00635	6.3	6.92	8.38	17.5
LL1397	3.30	3.87	14.66	0.00599	0.00623	3.9	8.61	9.44	8.8
Moderately tolerant genotypes									
LL1369	1.47	2.53	42.11	0.00445	0.00499	10.8	6.12	8.23	25.6
LL1385	1.76	2.43	27.70	0.00467	0.00580	19.5	6.40	9.15	30.0
Sensitive genotypes									
PL406	1.80	3.13	42.60	0.00460	0.00600	22.7	3.95	5.59	29.2
LL931	2.33	3.70	36.90	0.00480	0.00530	9.7	5.13	8.15	37.0
Highly sensitive genotypes									
IPL327	1.05	1.93	45.70	0.00442	0.00583	24.2	2.24	5.87	61.9
LL1365	0.63	1.63	61.20	0.00556	0.00798	30.4	2.38	6.32	62.4
Overall mean*	**1.76**	**2.64**	**33.30**	**0.00500**	**0.00610**	**17.3**	**5.14**	**7.57**	**32.1**
CD (5%)	**Treatments (T)**		**0.06**			**0.00012**			**0.10**
	Genotypes (G)		**0.25**			**0.00046**			**0.37**
	Interaction (T x G)		**0.35**			**0.0007**			**0.52**

*: Mean of 30 genotypes; ITP: Imazethapyr treated plots, CP: control plots; NS: non-significant

Table 5. Means value of some tolerant, moderately tolerant, sensitive and highly sensitive genotypes for pods plant^{-1}, 100-seed weight and seed yield in treated and control plots

Genotypes	Pods plant^{-1}			100-seed weight (g)			Seed yield (kg ha^{-1})		
Tolerant genotypes									
Genotypes	**ITP**	**CP**	**% Reduction**	**ITP**	**CP**	**% Reduction**	**ITP**	**CP**	**% Reduction**
EC78452	75.0	91.7	18.2	1.48	1.55	4.3	1167	1375	15.1
IPL406	27.7	33.0	16.2	3.20	3.24	1.2	1027	1207	14.9
LL699	67.7	73.7	8.1	1.77	1.84	3.8	1230	1360	9.6
LL1203	53.7	63.7	15.7	2.25	2.32	3.0	1044	1279	18.4
LL1397	65.7	79.3	17.2	1.50	1.57	4.7	1310	1482	11.6
Moderately tolerant genotypes									
LL1369	26.3	37.7	30.1	2.27	2.62	13.1	924	1279	27.8
LL1385	43.3	55.3	21.7	1.76	2.14	17.7	966	1360	28.9
Sensitive genotypes									
PL406	44.0	62.3	29.4	3.2	3.24	1.2	612	864	29.1
LL931	45.3	67.7	33.0	2.21	2.46	10.2	789	1248	36.8
Highly sensitive genotypes									
IPL327	15.7	31.0	49.5	1.86	2.35	21.0	347	877	60.4
LL1365	19.3	40.3	52.1	1.58	2.06	23.0	317	968	67.2
Overall mean*	**39.2**	**53.9**	**27.3**	**1.87**	**2.12**	**11.9**	**782**	**1155**	**32.3**
CD (5%)	**Treatments (T)**		**2.4**			**0.006**			**26**
	Genotypes (G)		**9.1**			**0.025**			**104**
	Interaction (T x G)		**NS**			**0.035**			**147**

*: Mean of 30 genotypes; ITP: Imazethapyr treated plots, CP: control plots; NS: non-significant

Table 6. Effect of different herbicides on weed population and weed dry weight in lentil

Treatment	Weed population m^{-2}				Weed dry weight ($g\ m^{-2}$)
	Oenothera drumundii **(Railway creeper)**	***Cyperus rotundus*** **(Purple nut sedge)**	***Medicago denticulata*** **(Toothed bur clover)**	***Lepidium sativum*** **(Pepper grass)**	
Imazethapyr 0.75 kg/ha	4.31 (17.83)*	2.05 (3.67)	1.91 (3.33)	4.20 (17.67)	14.23
Two Hand weeding 30 and 60 DAS	1.50(2.00)	1.00 (0.0)	1.00 (0.0)	3.42 (11.00)	9.03
CD (5%)	0.60	0.44	0.55	0.70	2.91

*Figures in parentheses are means of original values. Values after square root transformation.

Fig. 1.Variation for Imazethapyr herbicide tolerance in lentil genotypes

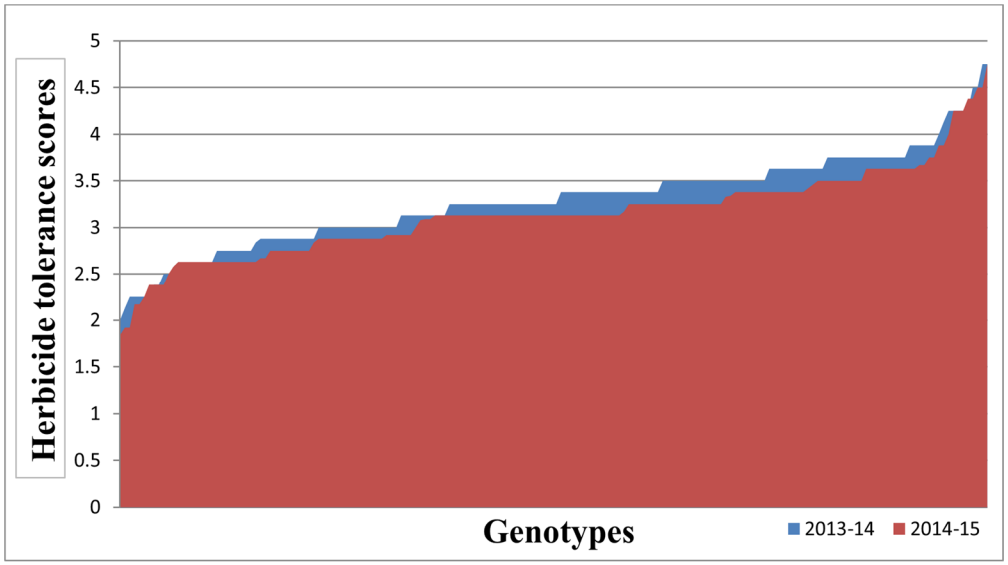

Fig. 2. Range of variation in 180 lentil genotypes for tolerance to herbicide Imazethapyr