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Article

Large Genetic Variability in Chickpea for Tolerance to Herbicides Imazethapyr and Metribuzin

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Abstract: Chickpea (*Cicer arietinum* L.) is known to be sensitive to many herbicides and, therefore, choices for using post-emergence herbicides for weed control are limited. The present study was aimed at identifying sources of tolerance to two herbicides with different modes of action (imazethapyr—amino acid synthesis inhibitor; and metribuzin—photosynthesis inhibitor) for use in breeding herbicide tolerant cultivars. Screening of 300 diverse chickpea genotypes (278 accessions from the reference set and 22 breeding lines) revealed large genetic variations for tolerance to herbicides imazethapyr and metribuzin. In general, the sensitivity of the genotypes to metribuzin was higher compared to that for imazethapyr. Several genotypes tolerant to metribuzin (ICC 1205, ICC 1164, ICC 1161, ICC 8195, ICC 11498, ICC 9586, ICC 14402 ICC 283) and imazethapyr (ICC 3239, ICC 7867, ICC 1710, ICC 13441, ICC 13461, ICC 13357, ICC 7668, ICC 13187) were identified, based on average herbicide tolerance scores from two experimental locations each. The herbicide tolerant lines identified in this study will be useful resources for development of herbicide tolerant cultivars and for undertaking genetic and physiological studies on herbicide tolerance in chickpea.

Keywords: chickpea; *Cicer arietinum*; genetic variability; herbicide tolerance; imazethapyr; metribuzin

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume, and is presently grown in more than 50 countries and imported by more than 150 countries [1]. It is the second largest grown and produced food legume after dry beans. During 2011, chickpea was grown on 13.2 million ha and about 80% of this area was in South and Southeast Asia (India 68%, Pakistan 8.9% and Myanmar 2.3%) [1]. The other major chickpea growing countries include Turkey, Australia, Ethiopia, Iran, Mexico, Canada and USA.

Chickpea is a poor competitor to weeds because of slow growth rate at early stages of crop growth and establishment [2]. Weeds compete with chickpea plants for water, nutrients, sunlight, and space and also harbor insect-pests and diseases. If left uncontrolled, weeds can reduce chickpea yield significantly. Weeds have become a serious problem in both autumn-sown and winter-grown chickpeas [2,3] and also if the crop is grown under irrigated conditions [4]. Significant yield losses (up to 84%) due to weeds have been reported in chickpea [5–9], more severely (up to 98%) in autumn-sown chickpea [2]. Thus, weed management is crucial in chickpea to realize maximum yields and also to maintain high quality of produce.

Hand weeding and mechanical weed control methods traditionally followed in the developing countries are becoming expensive due to increased labor wages. Because of the sensitivity of chickpea to herbicides, most effective are the pre-emergence herbicides, and choices for post-emergence herbicides are limited [2]. 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 loses. Therefore, chickpea cultivars with improved herbicide tolerance, which can offer greater flexibility for use of post-emergence herbicides, are required by the farmers.

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, e.g., a variety "Coromup" in narrow-leaf lupin [10] and a variety "Tracy-M" in soybean [11] with improved tolerance to 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 [12]. Similarly in lentils, a variety "RH44" with tolerance to imidazolinone was developed by mutagenesis [13]. There are few reports on identification of herbicide tolerance in chickpea [14,15]. However, these reports indicate presence of genotypic variations for herbicide tolerance in chickpea.

This study was aimed at identifying sources of tolerance to herbicides imazethapyr (amino acid synthesis inhibitor) and metribuzin (photosynthesis inhibitor) by screening a diverse set of germplasm so that these could be utilized for development of herbicide tolerant cultivars.

2. Results

Screening of 300 chickpea genotypes (278 genotypes from the reference collection and 22 cultivars/advanced breeding lines) for tolerance to two herbicides, metribuzin and imazethapyr, revealed large genetic variation for tolerance to these herbicides. On a scale from 1 to 5 (1 = highly tolerant and 5 = highly sensitive), the level of herbicide tolerance ranged from 1.50 to 5.00 for metribuzin and 2.00 to 5.00 for imazethapyr. It was observed that many lines showed susceptibility to both the herbicides and very few expressed tolerance to either or both the herbicides. In general, the sensitivity for metribuzin was higher as compared to that for imazethapyr (Figures 1 and 2). Based on the average scores of two locations, the most tolerant lines to each herbicide were identified (Table 1). The herbicide tolerance scores for these tolerant lines ranged from 1.50 to 2.88 for metribuzin and from 2.00 to 2.50 for imazethapyr. A few genotypes were found moderately tolerant to both herbicides (Table 1). There were several genotypes highly sensitive to both the herbicides. The five most sensitive genotypes to both the herbicides (scores 4.50 to 4.75 for metribuzin, and 5.00 for imazethapyr) are listed in Table 1.

Figure 1. Range of variation in chickpea genotypes studied for tolerance to herbicide metribuzin.

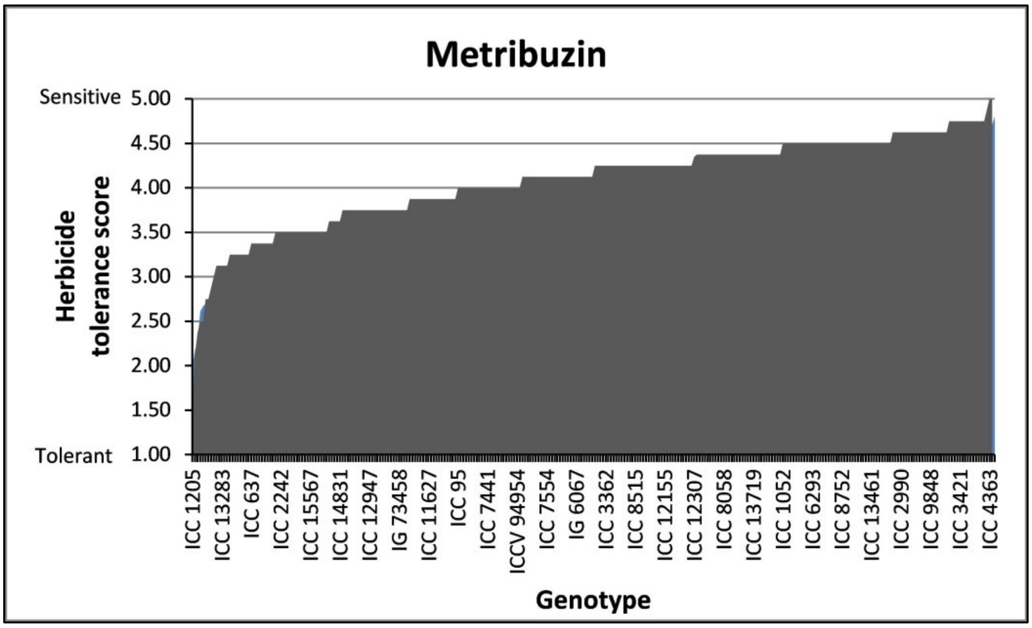

Figure 2. Range of variation in chickpea genotypes studied for tolerance to herbicide imazethapyr.

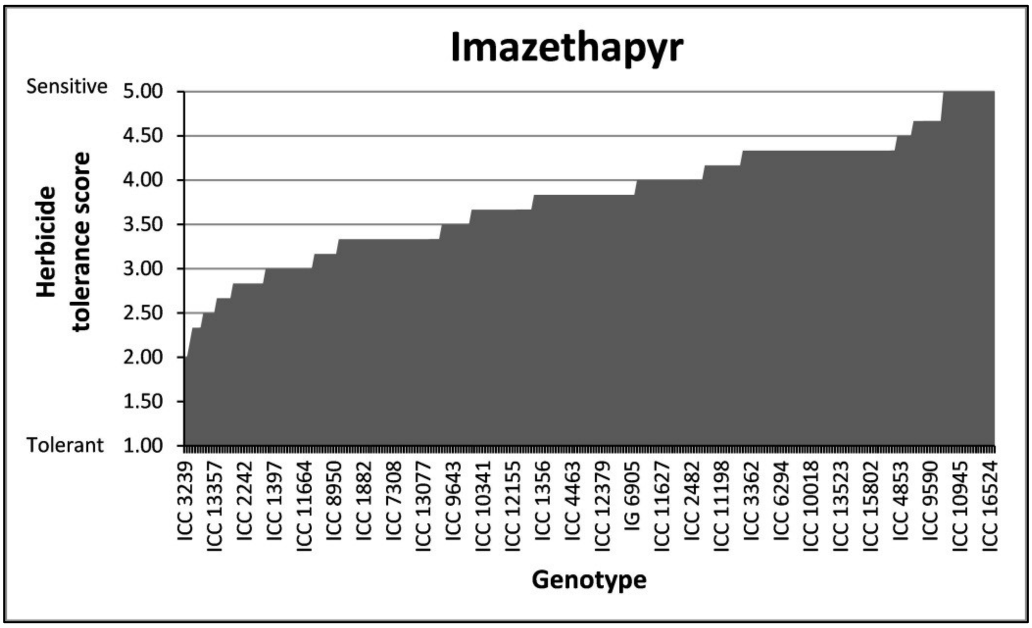

Table 1. Top ranking tolerant and sensitive chickpea genotypes for herbicides metribuzin and imazethapyr (Mean score ± SE on a scale from 1 to 5, where 1 = highly tolerant and 5 = highly sensitive).

Genotype	Metribuzin			Imazethapyr		
	IARI	PAU	Average	ICRISAT	IIPR	Average
Tolerant to metribuzin						
ICC 1205	1.50 ± 0.50	1.50 ± 0.00	1.50	3.67 ± 0.33	3.00 ± 0.50	3.34
ICC 1164	2.50 ± 0.50	1.75 ± 0.25	2.13	3.67 ± 0.33	3.00 ± 0.50	3.34
ICC 1161	2.50 ± 0.50	2.25 ± 0.25	2.38	2.67 ± 0.33	3.00 ± 1.00	2.84
ICC 8195	3.50 ± 0.50	1.50 ± 0.00	2.50	3.34 ± 0.67	4.00 ± 1.00	3.67
ICC 11498	3.50 ± 0.50	1.50 ± 0.00	2.50	3.00 ± 0.33	4.00 ± 0.50	3.50
ICC 9586	3.50 ± 0.50	2.00 ± 0.50	2.75	4.67 ± 0.33	2.00 ± 0.00	3.34
ICC 14402	3.00 ± 0.00	2.50 ± 0.50	2.75	4.67 ± 0.33	4.00 ± 0.50	4.34
ICC 283	3.50 ± 0.50	2.25 ± 0.25	2.88	4.67 ± 0.33	4.00 ± 1.00	4.34
Tolerant to imazethapyr						
ICC 3239	5.00 ± 0.00	3.50 ± 0.50	4.25	2.00 ± 0.33	2.00 ± 0.00	2.00
ICC 7867	5.00 ± 0.00	3.50 ± 0.50	4.25	2.34 ±0.33	2.00 ± 0.00	2.17
ICC 1710	5.00 ± 0.00	2.25 ± 0.25	3.63	2.67 ± 0.33	2.00 ± 0.00	2.34
ICC 13441	5.00 ± 0.00	2.75 ± 0.25	3.88	2.67 ± 0.33	2.00 ± 0.00	2.34
ICC 13461	5.00 ± 0.00	4.00 ± 0.00	4.50	2.67 ± 0.33	2.00 ± 0.00	2.34
ICC 13357	4.50 ± 0.50	3.00 ± 0.50	3.75	3.00 ± 0.00	2.00 ± 0.00	2.50
ICC 7668	5.00 ± 0.00	3.00 ± 0.50	4.00	3.00 ± 0.50	2.00 ± 0.00	2.50
ICC 13187	5.00 ± 0.00	3.75 ± 0.25	4.38	3.00 ± 0.00	2.00 ± 0.00	2.50
Tolerant to metribuzin and imazethapyr						
ICC 1161	2.50 ± 0.50	2.25 ± 0.25	2.38	2.67 ± 0.33	3.00 ± 1.00	2.84
ICC 1398	3.50 ± 0.50	3.00 ± 0.00	3.25	3.67 ± 0.33	2.00 ± 0.00	2.84
ICC 4841	3.50 ± 0.50	3.25 ± 0.25	3.38	3.00 ± 0.00	3.00 ± 1.00	3.00
ICC 7272	3.50 ± 0.50	3.50 ± 0.50	3.50	3.34 ± 0.33	2.00 ± 0.00	2.67

Table 1. *Cont.*

Genotype	Metribuzin			Imazethapyr		
	IARI	PAU	Average	ICRISAT	IIPR	Average
Sensitive to metribuzin and imazethapyr						
ICC 4918	5.00 ± 0.00	4.00 ± 0.00	4.50	5.00 ± 0.00	5.00 ± 0.50	5.00
ICC 5434	5.00 ± 0.00	4.00 ± 0.00	4.50	5.00 ± 0.00	5.00 ± 0.00	5.00
ICC 8318	5.00 ± 0.00	4.00 ± 0.00	4.50	5.00 ± 0.00	5.00 ± 0.50	5.00
ICC 15518	5.00 ± 0.00	4.00 ± 0.00	4.50	5.00 ± 0.00	5.00 ± 0.50	5.00
ICC 14077	5.00 ± 0.00	4.50 ± 0.00	4.75	5.00 ± 0.00	5.00 ± 0.50	5.00

Herbicides affected the vegetative growth of the susceptible lines. Metribuzin showed adverse effects on the lower leaves by causing necrosis followed by senescence. In highly susceptible genotypes, it caused complete death of the plants. Imazethapyr mainly killed the growing tips (epical meristem and young leaves) of the branches. Death of the plants was observed in highly sensitive genotypes. In moderately tolerant and tolerant genotypes, the death of epical meristem caused by imazethapyr induced branching. The effects were similar to that of the removal of shoot tips (called nipping), a general practice for inducing branching in chickpea. For both the herbicides, the most sensitive lines had 80% to 100% mortality (for example Line number 2 in Figure 3). Other abnormalities included elongation of branches (similar to tendrils) with very small or needle shaped leaves, delaying of flowering, deformation of flowers, poor pod setting and reduction in pod and seed size. Secondary growth was observed in many lines 20–25 days after herbicidal application leading to flowering and pod set.

Figure 3. Variation for herbicide tolerance in chickpea. Left to right Line #1 (score 3.0), Line #2 (score 5.0), Line #3 (score 4.0), Line #4 (score 2.0) and Line #5 (score 2.0).

The response of 11 tolerant to moderately tolerant (score 2–3) and 3 highly sensitive (score 5) genotypes for resistance to imazethapyr was confirmed by repeating screening of these genotypes at IIPR-Kanpur (Table 2). The herbicide tolerance scores in the repeat experiment were almost similar to those obtained in the first experiment.

Table 2. Comparison of herbicide tolerance scores from the two experiments conducted at IIPR, Kanpur (Mean score ± SE on a scale from 1 to 5, where 1 = highly resistant and 5 = highly sensitive).

S No	Genotype	Herbicide tolerance score	
		Expt 2	Expt 1
1	ICC 2990	3.0 ± 1.0	3.0 ± 0.5
2	ICC 1161	2.0 ± 0.0	3.0 ± 1.0
3	ICC 1164	2.0 ± 0.5	3.0 ± 0.5
4	ICC 2629	3.0 ± 0.0	3.0 ± 0.5
5	ICC 1205	3.0 ± 1.0	3.0 ± 0.5
6	ICC 1392	3.0 ± 0.0	2.0 ± 0.0
7	ICC 1356	3.0 ± 0.0	3.0 ± 0.5
8	ICC 5878	3.0 ± 1.0	2.0 ± 0.0
9	ICC 1397	3.0 ± 1.0	2.0 ± 0.0
10	ICC 13816	2.0 ± 0.0	2.0 ± 0.0
11	ICC 1710	2.0 ± 0.5	2.0 ± 0.0
12	ICC 5434	5.0 ± 0.0	5.0 ± 0.0
13	ICC 8522	5.0 ± 0.5	5.0 ± 0.5
14	ICC 10945	5.0 ± 0.0	5.0 ± 0.5

In the present study, several monocot and dicot weeds were observed in the field but dicot weeds were predominant. The effect of herbicidal application on weeds was also recorded. The data on weed population were used to identify dominant weed species at three locations: ICRISAT-Patancheru, IARI-New Delhi and PAU-Ludhiana. The dominant weed species observed at Patancheru, were *Cirsium* spp., *Convolvulus arvensis* L., *Medicago lupulina* L. and *Parthenium hysterophorus* L. The density of these weed species ranged from 3 to 8 plants/m^2. The highest density (8 plants/m^2) was for *Convolvulus arvensis* L. Other weed species recorded at ICRISAT-Patancheru, were *Cyperus rotundus* L., *Vicia sativa* L., *Tribulus terrestris* L., *Euphorbia* spp., *Amaranthus sp.*, *Chenopodium album* L. and *Cardiospermum halicacabum* L. Similarly, the dominant species identified at IARI-New Delhi were *Convolvulus arvensis* L, *Chenopodium murale* L., *Cyperus rotundus* L. and *Fumaria parviflora* Lam. The density of these weed species ranged from 3.3 to 9.1 plants/m^2. *Chenopodium murale* L. recorded the highest density of 9.1 plants/m^2. Thus, *Chenopodium* spp. were found common in chickpea fields at both the locations. Based on data recorded at ICRISAT-Patancheru on weed numbers, both pre- and post-emergence application of imazethapyr controlled 75%–80% of major dominant weeds. The treatment was very effective especially on *Cirsium* spp., and *Parthenium hysterophorus* L. At PAU-Ludhiana, the major weed (about 98% of total weeds) found was railway creeper (*Oenothera drumundii* Hook), a broad-leaf weed. The tested herbicide (metribuzin) controlled this weed effectively.

3. Discussion

This study revealed large genetic variations for tolerance to herbicides metribuzin and imazethapyr in chickpea. The genotypes screened included 93% of the genotypes of chickpea reference collection, which consists of 300 genotypes. Upadhyaya *et al.* [16] reported that the chickpea reference collection represented variability present in the genetic resources available at ICRISAT and International Center for Agricultural Research in Dry Areas (ICARDA). The reference collection was developed from the composite collection of chickpea based on allelic diversity data [16]. The composite collection consists of 3000 accessions and includes 1956 accessions of ICRISAT core collection, 709 accessions from the genebank of ICARDA, 39 advanced breeding lines and cultivars, 241 trait-specific accessions (resistant/tolerant to biotic and abiotic stresses, early maturity, multi-seeded pods, double podded, large-seed size, high seed protein, high nodulation, and responsive to high input conditions) and 20 accessions of two wild *Cicer* species (*C. echinospermum* Davis and *C. reticulatum* Lad.) [17]. The reference collection is reported to have captured 78% of the 1791 alleles of the composite collection [16].

Herbicide tolerance ratings based on plant injury on a 0–9 scale was reported to be rapid and reliable measurement of tolerance to herbicides in chickpea [14]. We further simplified this scale and found that a 1–5 scale is fairly reliable and convenient in measuring herbicide tolerance in chickpea.

The experiment repeated at IIPR-Kanpur with selected genotypes showed good correspondence in the herbicide tolerance scores from the two screenings. This indicates the robustness of the screening methodology followed in the present study to identify sources of herbicide tolerance in chickpea. Among the top eight tolerant genotypes identified for each herbicide, no genotype was tolerant to both the herbicides. This may be due to differences in the genes controlling tolerance to these herbicides which have different modes of action—imazethapyr inhibits amino acid synthesis, while metribuzin inhibits photosynthesis.

Data recorded on weed species killed by these herbicides indicated that the weed control was effective by the tested herbicide at these locations. These results suggest that the post-emergence application of these herbicides by developing herbicide tolerant cultivars will be an effective strategy for weed management in chickpea.

Imazethapyr, an imidazolinone (IMI; includes "Imazethapyr") 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 chickpea and other legume fields [18]. They provide flexibility in application time, lower rates of application and have low mammalian toxicity [19]. 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 [20]. After being translocated through phloem, it inhibits ALS resulting in the death of meristematic cells and finally the whole plant [21]. The imidazolinone group of herbicides are registered in Turkey for the control of broadleaf weeds in chickpea [22]. A recent study showed the existence of wide range of natural variation for tolerance to IMI group of herbicides (imazethapyr and imazamox) in chickpea [14]. Under glasshouse conditions, three chickpea genotypes ICC 2242, ICC 2580 and ICC 3325 were found common and showed very low rating of herbicide injury (2 = good plant appearance with minor chlorosis or leaf curling). These genotypes were also included in the present study, and found as moderately tolerant to both metribuzin (3.5–3.75) and imazethapyr (3.14–3.72). These differences could possibly be due to differences in growing environments used in screening the genotypes. Genetic variation for tolerance to imidazolinone class of herbicides has also been observed in field pea [23].

Efforts have also been made to induce resistance to imidazolinone class of herbicides in chickpea by induced mutagenesis through gamma rays [24]. Nine accessions of chickpea belonging to three species (*C. arietinum* L., *C. reticulatum* Lad. and *C. bijugum* K.H. Rech.) were treated. One mutant with high levels of resistance to imidazolinone was identified in *C. reticulatum* Lad. This study suggests that induced mutagenesis is a potential method of inducing herbicide tolerance in chickpea.

The other herbicide used in this study was metribuzin which controls weeds through inhibiting the photosynthesis *i.e.*, photosystem II. The pre-emergence application of metribuzin was reported to be effective against *Chenopodium* spp. and seed yields of chickpea under metribuzin treatment were similar to clean-weeded plot [5]. Metribuzin tolerant mutant lines have been identified in narrow-leaf lupin [25]. Two tolerant chickpea accessions (IG 96220 and S98167-CLIMA) were identified after screening 100 germplasm accessions from across the world [15]. Recent study conducted at Centre for Legumes in Mediterranean Agriculture (CLIMA), Australia has shown that metribuzin can be used as a post-emergence herbicide to control broad-leaf weeds in chickpea [15].

A few herbicides have been registered officially for weed control in chickpea in Australia, Turkey and Canada. In Australia, isoxaflutole (75% a.i.) at 100 g ha^{-1} is registered for the control of several broadleaf weeds in chickpea [26]. In Turkey, herbicides such as methabenzthiazuron, terbutryne and imazethapyr have been registered and authorized for weed control in chickpea. Similarly, metribuzin has been registered for use in chickpea in Canada [27]. No post-emergence herbicide has been recommended for weed control in chickpea in South Asia where bulk of chickpea is grown. This is mainly because the available chickpea cultivars are sensitive to herbicides.

The genetics of herbicide tolerance has been studied in some legume crops. Inheritance of soybean response to a few herbicides has been characterized. A single recessive gene was found to control metribuzin sensitivity [28]. A small number of genes exhibiting partial dominance were responsible for resistance to propanil injury [29], whereas a complex pattern of inheritance was observed for pendimethalin injury in soybeans [30]. Sulfentrazone tolerance was attributed to a single dominant gene in two soybean cultivars [31]. In *Medicago* spp. segregation ratios indicated the control of sulfonylurea (SU) herbicide tolerance by a single dominant gene [32]. Similarly, a single partially dominant gene was also reported to impart resistance to metribuzin in narrow-leaf lupin [33].

Herbicide tolerant chickpea 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 chickpea 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.

Breeding for herbicide tolerant cultivars has also been initiated by the institutes participating in this study. A few most tolerant genotypes for each herbicide identified in this study have been crossed with selected high yielding cultivars. The ultimate goal is to develop cultivars with herbicide tolerance, high yield potential, desired maturity duration, resistance to key diseases and market-preferred seed quality. We hope that these efforts would lead to development of herbicide tolerant cultivars in chickpea in the near future.

4. Experimental Section

A total of 300 chickpea genotypes were used in this study. These included 278 genotypes from the reference collection and 22 cultivars/advanced breeding lines developed at the International Crops

Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru. The complete reference collection (*n* = 300) could not be included in this study because adequate quantity of seed was not available for 22 accessions (all accessions of wild species and few accessions of the cultivated species). The genotypes studied originated from 30 countries and over 80% of the accessions were landraces (Table 3). According to the types of chickpea, 69% were desi type (colored seed coat), 28% kabuli type (white seed coat) and 3% intermediate type (pea-shaped seed).

The experiment was conducted during post-rainy season 2011–2012 at four locations in India—ICRISAT, Patancheru (17°52' N, 78°24' E); Indian Institute of Pulses Research (IIPR), Kanpur (26°28' N, 80°24' E); Indian Agricultural Research Institute (IARI), New Delhi (28°36' N and 77°12' E); and Punjab Agricultural University (PAU), Ludhiana (30°55' N, 75°54' E). The screening for tolerance to metribuzin was carried out at IARI-New Delhi and PAU-Ludhiana and for imazethapyr at ICRISAT-Patancheru and IIPR-Kanpur. Sowing was done during 2011 on 2 October at ICRISAT-Patancheru, 28 September at IIPR-Kanpur, 5 November at IARI-New Delhi, and 2 December at PAU-Ludhiana. The experiment was conducted in the field in a randomized block design (RBD) with 3 replications at ICRISAT-Patancheru and 2 replications at other locations. The plants were grown on 1-m long rows (2-m rows at IIPR Kanpur) with 30 cm spacing between rows and 10 cm between plants.

An experiment was initially conducted to identify optimum rate for herbicide application. Three rates, 50%, 100% and 150% of the recommended rate given on the label, were used. We found that the rate given on the label recommendation is effective in controlling most of the weeds and differentiating chickpea genotypes for herbicide tolerance. Thus, as per the label recommendation the herbicides metribuzin (Sencor: Bayer) and imazethapyr (Pursuit: BASF) were uniformly sprayed at the rate of 250 g ha^{-1} and 750 mL ha^{-1} respectively, 30-days after sowing using a shoulder-mounted hand operated knapsack sprayer. The spray solution was sprayed at 375 L ha^{-1} for both herbicides. For uniform spread and absorption, imazethapyr was mixed with Cyboost (700 g per ha) and Cyspread (565 mL per ha) in the spray solution. The spraying was done during cooler hours of the day when there was little or no wind. The plants were scored for herbicide tolerance 2-weeks after spray on a 1–5 scale; where 1 = highly tolerant (excellent plant appearance, no burning/chlorosis of leaves), 2 = tolerant (good plant appearance with minor burning/chlorosis of leaves), 3 = moderately tolerant (fair plant appearance with moderate burning/chlorosis of leaves), 4 = sensitive (poor plant appearance with severe burning/chlorosis of leaves), and 5 = highly sensitive (complete burning of leaves leading to plant mortality). The average scores from different replications were used to identify tolerant and sensitive lines.

No weeding was done prior to or post herbicidal application. Observations on weeds were recorded at three locations, ICRISAT-Patancheru, IARI-New Delhi and PAU-Ludhiana. The dominant weed species were identified at these locations. The data were recorded on kinds and number of weeds before and after the herbicide spray. The density of different weeds per m^2 was determined from 10 locations in the experimental field.

A set of 14 lines (11 tolerant + 3 sensitive) from the first experiment (described above) was again screened at IIPR-Kanpur for tolerance to imazethapyr to verify the herbicide tolerance reaction observed in the first experiment. This second experiment was planted on 22 November 2011. The experimental procedure was similar to those explained above.

Table 3. General information on chickpea genotypes used for herbicide tolerance screening.

General information	Genotypes of reference set	Breeding lines/cultivars	Total
Type of seed			
Desi type	193	13	206
Kabuli type	75	9	84
Pea-shaped	10	-	10
Total	278	22	300
Biological status			
Recent cultivars/breeding lines	15	22	37
Land races/old cultivars	258	-	258
Unknown pedigree	5	-	5
Total	278	22	300
Country of origin			
Afghanistan	16	-	16
Algeria	2	-	2
Bangladesh	1	-	1
Chile	2	-	2
China	1	-	1
Cyprus	3	-	3
Egypt	1	-	1
Ethiopia	14	-	14
Germany	1	-	1
Greece	1	-	1
India	92	22	114
Iran	76	-	76
Iraq	1	-	1
Israel	2	-	2
Italy	3	-	3
Malawi	3	-	3
Mexico	4	-	4
Morocco	6	-	6
Myanmar	2	-	2
Nepal	2	-	2
Nigeria	1	-	1
Pakistan	6	-	6
Peru	1	-	1
Portugal	1	-	1
Sudan	1	-	1
Syria	10	-	10
Tanzania	2	-	2
Turkey	13	-	13
USSR	6	-	6
Unknown	3	-	3
USA	1	-	1
Total	278	22	300

5. Conclusions

The large genetic variability identified in chickpea for herbicide tolerance will stimulate further research efforts towards development of herbicide tolerant cultivars. The reference set provided a good indication of the variability present in the germplasm for herbicide tolerance and it would encourage further screening of a greater germplasm collection to search for even more robust and diverse sources of herbicide tolerance. The herbicide tolerant genotypes identified in this study would be useful in genetic and physiological studies and in development of herbicide tolerant cultivars. Crosses have been attempted between the most contrasting lines for herbicide tolerance to develop recombinant inbred lines (RILs) at ICRISAT-Patancheru and IIPR-Kanpur. These will be used to identify molecular markers linked to gene(s) for herbicide tolerance. Marker-assisted selection for herbicide tolerance will greatly improve precision and efficiency of breeding for herbicide tolerance. Excellent progress has been made in the recent past in development of genomic resources for chickpea [34]. The availability of the sources of herbicide tolerance and vast genomics resources would facilitate rapid progress in the development of herbicide tolerant chickpea cultivars.

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Conflicts of Interest

The authors declare no conflicts of interest in this work.

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