Indian J. Genet., 77(3): 400-407 (2017)
DOI: 10.5958/0975-6906.2017.00054.2

Tolerance to post-emergence herbicide Imazethapyr in chickpea

Nitish Ranjan Prakash, Rajesh Kumar Singh, S. K. Chauhan, Mukesh K. Sharma, C. Bharadwaj, V. S. Hegde, P. K. Jain[1], P. M. Gaur[2] and Shailesh Tripathi*

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi110 012; [1]National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110 012; [2]International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324

(Received: January 2017; Revised: July 2017; Accepted: July 2017)

Abstract

The present research work aimed at identification of sources of tolerance to herbicide Imazethapyr for their possible utilization in development of herbicide tolerant chickpea. Sixty five genotypes (55 *desi* and 10 *kabuli*) screened included accessions from ICRISAT core collection, advanced breeding lines and cultivars. The herbicide tolerance score ranged from 1.9 to 5.0. Nine tolerant to moderately tolerant and three susceptible genotypes were further evaluated under control and sprayed condition. Genotype x environment interactions were observed for days to 50% flowering, NDVI, days to maturity, seed yield, biomass, harvest index, 100-seed weight and branched chain amino acids (BCAA) *viz*., valine, leucine and isoleucine content. Highly significant reduction in seed yield was observed in all the genotypes except ICCV 10, ICCL 82104 and ICC 1710 as revealed by pairwise comparison of means using Tukey's test. The spraying of herbicide reduced the total biomass production. Analysis of BCAA content in sample revealed non-significant differences for percent valine content in ICCIL 04001, ICCV 00305, ICCV 96003 and ICCL 82104, for isoleucine content in all the genotypes except, ICCV 3 and ICCV 96003 and for leucine content in case of ICCV 03407, ICCIL 04001, ICCV 10, ICCV 96003, ICC 1710, ICCV 00108 and ICCL 82104. The genotypes tolerant to post-emergence herbicide Imazethapyr identified based on non-significant reduction in the yield attributes and BCAA content in the sample were ICC 82104, ICCV 10, ICCV 96003, ICC 00305 and ICC 1710. These genotypes can be used to study the genetics of herbicide tolerance in chickpea and in breeding programs for developing lines with tolerance to post-emergence herbicide Imazethapyr.

Keywords: Imazethapyr, variation, herbicide tolerance, Normalized Difference Vegetation Index, branched chain amino acids

Introduction

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after dry beans,grown in an area of ~14 mha with production of 13.7 mt (FAOSTAT 2014). The major chickpea producing countries contributing to about 95% of the global production include India, Turkey, Australia, Pakistan, Myanmar, Ethiopia, Canada, Iran and USA.Chickpea crop is highly affected by weeds during entire growth period. Most of the weed species grow faster and taller thanchickpea inhibiting growth, curtail sunlight, and ultimately affect photosynthesis and plant productivity. The critical weed control period in chickpea has been reported to be 30-60 days after emergence (Solh and Pala 1990) or 5-7 weeks after crop emergence in Mediterranean region (Al-Thahabi et al. 1994) or 4-5 leaf stage to flowering (Mohammadi et al. 2005). Yield loss incurred due to weed competition in chickpea is estimated to be between 40 to 87% depending on the type of weeds and their density (Bhan and Kukula 1987). Plew et al. (1994) reported less dry matter accumulation in chickpea due to weed competition at early stage of crop growth.

Shortage of agricultural labourers coupled with continuously increasing labour cost has made manual weeding an expensive field operation in any crop in a developing world. Herbicide-tolerant cultivars offer an opportunity of controlling weeds through need-based applications of herbicides thus bringing down the cost of cultivation. In chickpea, pre-emergence herbicides are commonly used to control weeds emerging soon after germination (Solh and Pala 1990). Weeds'

*Corresponding author's e-mail: shaitri@rediffmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; http://epubs.icar.org.in/journal/index.php/IJGPB

germinating after crop emergence still remains a major bottleneck in chickpea production. Post-emergence chemical weed control options are few in chickpea and the available herbicides have adverse effect on crop.

Imazethapyr, a post-emergence broad spectrum herbicide belonging to Imidazolinone group, is commonly used in pulse crops. Imazethapyr acts through inhibition of Aceto-Hydroxy Acid Synthase (AHAS) or Acetolactate synthase (ALS) enzyme which catalyzes the first step in synthesis of branched chain amino acids (valine, isoleucine and leucine) (Shaner et al. 1984; Duggleby and Pang 2000). Application of most of the ALS inhibiting herbicide results into reduced level of BCAA (valine, leucine and isoleucine) in the total free amino acid pool (Shaner et al. 1984). Biosynthesis of BCAA occurs generally in meristematic tissues; therefore young tissues are the first to show symptoms. Zhou et al. (2007) reported that resistance to AHAS inhibiting herbicide results from altered sequences of AHAS encoding genes leading to production of altered AHAS having tolerance to herbicide. Gaur et al. (2013) reported that imazethapyr mainly killed the meristematic tips of branches resulting in elongation of branches, needle shaped leaves, delayed flowering, deformation of flowers and bud, poor pod setting and reduction in pod and seed size.

Herbicide tolerant varieties have been released in narrow-leaf lupin (Coromup (Si et al. 2008)) and soybean (Tracy-M (Hartwig 1987)). Mergoum et al. (2009) at North Dakota State University (NDSU), USA developed a hard red spring wheat variety 'ND901CL' which was released in 2008.Variations for tolerance to herbicide is present in natural germplasm in different crops such as in sunflower for imazethapyr (Al-Khatib et al. 1998), in chickpea for imidazolinone group of herbicide (Ping 2009; Taran et al. 2010; Gaur et al. 2013; Chaturvedi et al. 2014) and for metribuzin (Gaur et al. 2013). Thomson and Taran (2014) carried out genetic analysis of a chickpea line tolerant to imidazolinone herbicide and concluded that there are two homologous AHAS genes namely *AHAS1* and *AHAS2* with almost 80% amino acid sequence similarity. They found a point mutation in the *AHAS1* gene at C675 to T675 leading to an amino acid substitution of Ala205 to Val205 conferring resistance to imidazolinone herbicides in chickpea. Similarly genes conferring resistance to imidazolinone group of herbicides have also been reported in other crops such as sunflower (Al-Khatib and Miller 2000;Kolkman et al. 2004), soybean (Walter et al. 2014) and barley (Lee et al. 2011). The present investigation was undertaken to identify the chickpea lines tolerant to herbicide imazethapyr and characterize the tolerant and susceptible lines for yield attributes and biochemical parameters.

Materials and methods

Experimental material

The screening of genotypes was done at research farm of Indian Agricultural Research Institute (IARI), New Delhi during post-rainy season 2014-15 with sixty five diverse *desi* (55) and *kabuli* (10) chickpea genotypes. The seeds were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and IARI. The material was planted in randomized complete block design (RCBD) with three replications in 2m row with row to row spacing of 60 cm. Spraying of Imazethapyr (Pursuit, BASF) @ 75g a.i./ha was done 40-45 days after sowing. Herbicide tolerance score was recorded visually on 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) at 10, 20 and 30 days after spray. The average of scores from different replications was used to identify most tolerant and sensitive lines.

Nine lines tolerant to moderately tolerant lines, namely, ICC 1710, ICCL 82104, ICCV 96003, ICCV 03407, ICCV 00305, ICCV 3, ICCV 10, ICCV 00109 and ICCIL 04001 and 3 highly sensitive genotypes viz., Pusa 5028, ICCV 97017 and ICCV 00108 selected from preliminary screening were further evaluated during crop season 2015-16 in RCBD with 3 replications under control and sprayed conditions. Recommended agronomic practices and crop management measures were followed to ensure good crop stand. Observations were recorded on Normalized Difference Vegetation Index (NDVI) at 20 days after spray, days to 50% flowering, days to maturity, plant height, biomass, seed yield, 100-seed weight and BCAA (valine, leucine and isoleucine) content at 20 days after spray.

NDVI, phenological and morphological data scoring

NDVI (Normalized Difference Vegetation Index) was scored with the help of instrument called *GreenSeeker*^{TM}. The instrument was passed over the foliage of each row after clicking the on button and by keeping a constant height of approximately 70 cm from the crop canopy while measuring. Days to 50% flowering was calculated from date of sowing to the date when 50% of the plants have at least one open flower. For plant height, height of randomly selected three plants in each line was measured with a metre scale from ground level to tip of the plant.Days to maturity was recorded from the date of sowing to the date when 80% of the plants in a line turn brown or brownish yellow.The plot biomass was measured at the time of harvest. The weight of above ground part of the plants, yield and 100-seed weight was measured using an electronic balance.

Branched Chain Amino Acid (BCAA) estimation

The leaf samples of twelve genotypes were collected from all three replications under control as well as sprayed condition. The samples were collected 20 days after spray for BCAA estimation. Hydrolysis of powdered leaf sample was done in a capped test tube by adding 100 mg of sample + 800 µl of 6N HCl + 100 µl of 0.1 N HCl + 100 µl of Nor-leucine standard + 10 µl of Phenol and incubated for 16 hours at 110^{o}C in dry bath. Neutralization of hydrolyzed sample was done after cooling to room temperature (RT). Hydrolysates were then transferred to 10 ml measuring flask and 500 µl of 12.5 M NaOH was added. Volume was made upto 10 ml by adding 0.1N HCl. The mixture was filtered through 0.22 µ nylon filter and stored in a 2 ml tube. Pre-column derivatization was done with 100 µl of sample. Sample was then transferred to a 10 ml tube and 900 µl of Boric Acid (pH 6.2) + 1 ml of FMOC (100mg in 100ml acetone) were added. It was vortexed till the precipitate dissolved completely. Tube was then incubated for 5 minutes at RT. 4 ml of n-Pentane was then added to it and vortexed to mix it completely. It was again incubated for 5 minutes at RT. The upper layer was discarded and the bottom layer containing the sample was poured into UPLC vial for analysis.The chromatography column used was (Acclaim C18, 250 X 4.6, 5µ) from Thermo Fisher Scientific. Flow rate was kept at 1 ml per minute at 25^{o}C. Different mobile phases (A-1800 ml of Buffer + 200 ml of Organic phase and B-200 ml of Buffer + 1800 ml of Organic phase) were used during the analysis. Buffer used were prepared from 2.23 g of Tetra methyl Ammonium Chloride and 4.07 g of Sodium acetate trihydrate which was dissolved in HPLC grade water and volume made upto 2 liter, pH adjusted to 3.5 with acetic acid. Organic phase was made by mixing 1960 ml of HPLC grade Acetonitrile and 40 ml of Methanol.

Statistical analysis

One way ANOVA and two way ANOVA were performed. The standard error for difference (SE), LSD and Tukey's test were performed using PROC GLM of SAS. Tukey's test was used for pair-wise comparison of the treatment means:

$$q_s = \frac{(Y_a - Y_b)}{SE}$$

Where, Y_a and Y_b are the two means under comparison and SE is the standard error. The q_s value is compared with q value from studentized range distribution. If q_s is greater than critical value obtained from distribution, the two means are considered to be significantly different.

Results and discussion

Preliminary screening of genotypes for herbicide tolerance

Wide variation for tolerance to herbicide Imazethapyr was observed in the 65 genotypes screened. On a scale of 1-5 (1=highly tolerant and 5=highly sensitive), the overall average herbicide tolerance score for 65 chickpea lines ranged from 1.9 to 5.0 (Fig. 1). Gaur et

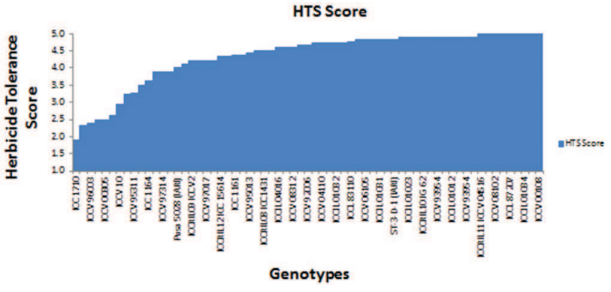

Fig. 1. Variability among sixty five genotypes for tolerance to herbicide Imazethapyr

al. (2013) also reported variation in herbicide tolerance from 1.5 to 5.0 for Metribuzin and 2.0 to 5.0 for Imazethapyr. Nine lines were found to be tolerant or moderately tolerant for Imazethapyr with average score less than 3.5. The response of nine tolerant to

moderately tolerant (ICC 1710, ICCL 82104, ICCV 96003, ICCV 03407, ICCV 00305, ICCV 3, ICCV 10, ICCV 00109 and ICCIL 04001) and three highly susceptible genotypes (Pusa 5028, ICCV 97107 and ICCV 00108) was confirmed by further evaluation of these lines under sprayed and control conditions.

Imazethapyr is a systemic herbicide which is absorbed by plant surface and once in the phloem it get translocated to the site of action, causing death of growing meristematic cells resulting in plant death. It is a slow acting herbicide and morphological symptoms starts to appear around 7-8 days after spray and hence, scoring after 10 days of spray is justifiable. Visual scoring for herbicide tolerance was done 10, 20 and 30 days after spray. Gaur et al. (2013) reported that new flushes start to come after 25 days of spray, therefore, one scoring should be done after 25 days to take into account plants which have recovered fast. Taran et al. (2010) scored the plant injury in greenhouse condition at 7, 14 and 21 days after spray. Herbicide tolerance score based on plant injury is fairly reliable in identifying tolerant and susceptible genotypes. In our study, we have followed a 1-5 scale (Gaur et al. 2013). Researchers have used different scales for herbicide tolerance screening *viz*., 0-7 scale by Chaturvedi et al. (2014) and 0-9 scale by Taran et al. (2010).

Effect of herbicide on phenology and yield attributes

Analysis of variance (ANOVA) revealed highly significant variation among genotypes for all the traits measured except NDVI under control condition and days to 50% flowering under sprayed condition. Similar results were obtained by Srivastava et al. (2011) and Malik et al. (2009). Significant to highly significant genotype x environment (G X E) interaction was observed for days to 50% flowering, NDVI, days to maturity, yield, biomass, harvest index, 100-seed weight and BCAA (valine, leucine and isoleucine) content (Table 1). Two way ANOVA revealed that almost all the traits were affected by spray of herbicide.

Days to 50% flowering under control condition was earliest in ICCV 03407 (52 days) and maximum in ICCV 97017 and ICC 1710 (77 days). Comparison of mean days to 50% flowering under control and sprayed condition revealed that flowering was delayed by almost one month under sprayed condition. Tukey's pair wise comparison of means for GxE revealed significant differences for days to 50% flowering in all the genotypes under control and sprayed conditions (Table 2). Delay in flowering as a result of herbicide spray has also been reported by Gaur et al. (2013). NDVI is a measurement of total plant growth that takes into account several plant growth factors. It is an indicator of photosynthetic activity of the plant. NDVI score differed significantly under control and sprayed condition with mean value of 0.661 and 0.447 respectively. Under sprayed condition, NDVI was highest in ICCV 96003 while lowest value was recorded in the genotype ICCL 82104 (Fig. 2). The overall mean NDVI score was significantly low under sprayed condition. This may be due to killing of the apical meristem and young leaves by Imazethapyr. Only one genotype, ICCV 96003 showed non-significant reduction in NDVI under sprayed condition.

Pooled analysis revealed significant differences in the mean plant

Table 1. Two-way ANOVA for morphological and biochemical traits under control and sprayed conditions (GxE)

Source of variation	df	MSS											
		DTF	NDVI	Height	DTM	Yield	Biomass	SW	HI	Valine	Isoleucine	Leucine
Replication	2	12.93056	0.00028	76.2	0.875	424.848	16057.743	1.991239	0.0071**	0.0000071	0.0005	0.000250
Environment		13805.681**	0.82347**	3698**	0.500	84781.38**	1886666.075**	408.79**	0.0187**	0.0054**	0.01914**	0.00328**
Genotype	11	105.802**	0.00781**	76.12**	76.89**	1004.53**	10594.991**	140.29**	0.0050**	0.00046**	0.00495**	0.00358**
G X E	11	107.923**	0.00817**	60.12	8.167*	1350.8**	12463.598**	30.64**	0.0085**	0.00017**	0.00309**	0.002014**
Error	46	12.2204	0.00217	23.92	4.778	100.79	3865.025	1.41	0.00176	0.000035	0.00033	0.00016
Total	71	235.8402	0.015485	90.8349	16.41549	1636.28	33101.65	33.21118	0.003684	0.000196	0.00174	0.001024

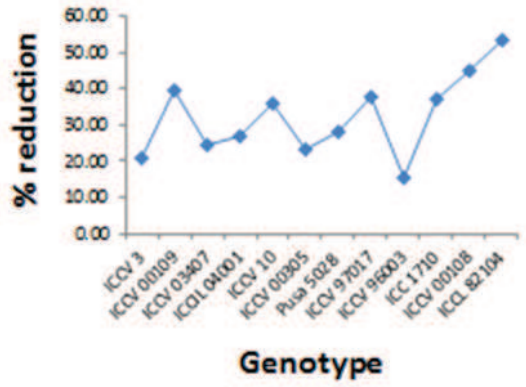

Fig. 2. Percent reduction in NDVI under sprayed condition

height under control and sprayed conditions. The reduction in plant height because of herbicide spray was minimum in the genotype ICCV 00109 (13%) and maximum in case of ICCL 82104 (54%). Mean days to maturity remained same under both control and sprayed conditions. This shows that though the spray of herbicide delayed flowering and onset of reproductive phase but the genotypes matured at the same time because of sudden rise in temperatures at maturity. None of the genotypes showed significant differences in mean days to maturity between the two environments. Average seed yield per plot of genotypes was 80.56g and 11.93g under control and sprayed conditions. Highly significant reduction in yield was observed in all the genotypes except ICCV 10, ICCL 82104 and ICC 1710 as revealed by pairwise comparison of means using Tukey's test. Maximum reduction in yield was observed in genotypes ICCV 97017 (98%) followed by Pusa 5028 (95%) (Fig. 3).

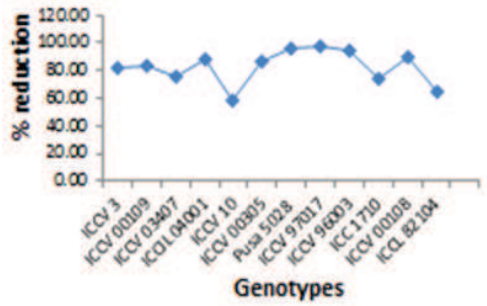

Fig. 3. Percent reduction in yield under sprayed condition

Taran et al. (2013) also observed that post-emergence application of Imazethapyr delayed flowering and maturity and reduced the yield in chickpea. The spraying of herbicide reduced the total biomass production. Average biomass per plot of genotypes

Table 2. Pairwise comparison of means under control and sprayed conditions

Genotype	NDVI		Seed yield (g)		Leucine (%)		Isoleucine (%)		Valine (%)	
	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
ICCV 3	0.653^{a}	0.517^{b}	90.48^{a}	16.83^{b}	0.084^{a}	0.132^{b}	0.261^{a}	0.128^{b}	0.0767^{a}	0.0447^{b}
ICCV 00109	0.677^{a}	0.41^{b}	100.57^{a}	16.26^{b}	0.095^{a}	0.150^{b}	0.157^{a}	0.167^{a}	0.0483^{a}	0.029^{b}
ICCV 03407	0.623^{a}	0.47^{b}	63.09^{a}	14.853^{b}	0.0743^{a}	0.1^{a}	0.164^{a}	0.111^{a}	0.0463^{a}	0.0277^{b}
ICCIL 04001	0.667^{a}	0.487^{b}	85.25^{a}	10.29^{b}	0.122^{a}	0.112^{a}	0.130^{a}	0.140^{a}	0.052^{a}	0.045^{a}
ICCV 10	0.663^{a}	0.427^{b}	57.79^{a}	23.79^{a}	0.087^{a}	0.099^{a}	0.147^{a}	0.093^{a}	0.0583^{a}	0.0283^{b}
ICCV 00305	0.657^{a}	0.503^{b}	81.75^{a}	11.20^{b}	0.067^{b}	0.137^{a}	0.094^{a}	0.141^{a}	0.0353^{a}	0.0357^{a}
Pusa 5028	0.68^{a}	0.49^{b}	122.50^{a}	5.59^{b}	0.138^{a}	0.095^{b}	0.173^{a}	0.139^{a}	0.060^{a}	0.039^{b}
ICCV 97017	0.667^{a}	0.417^{b}	110.4^{a}	2.78^{b}	0.064^{a}	0.123^{b}	0.113^{a}	0.073^{a}	0.0453^{a}	0.0233^{b}
ICCV 96003	0.66^{a}	0.557^{a}	60.57^{a}	3.76^{b}	0.124^{a}	0.117^{a}	0.171^{a}	0.113^{b}	0.0547^{a}	0.0527^{a}
ICC 1710	0.64^{a}	0.403^{b}	40.82^{a}	10.53^{a}	0.184^{a}	0.173^{a}	0.162^{a}	0.144^{a}	0.061^{a}	0.0417^{b}
ICCV 00108	0.693^{a}	0.38^{b}	110.2^{a}	12.07^{b}	0.132^{a}	0.103^{a}	0.135^{a}	0.084^{a}	0.0547^{a}	0.027^{b}
ICCL 82104	0.647^{a}	0.30^{b}	43.35^{a}	15.28^{a}	0.128^{a}	0.133^{a}	0.182^{a}	0.165^{a}	0.0603^{a}	0.0517^{a}
Mean	0.661^{a}	0.447^{b}	80.56^{a}	11.93^{b}	0.109^{b}	0.123^{a}	0.157^{a}	0.125^{b}	0.0544^{a}	0.0371^{b}
S.E (Genotype)	0.019		4.10		0.0052		0.0074		0.0024	
S.E. (Environment)	0.0078		1.673		0.002		0.003		0.00098	
S.E. (G X E)	0.027		5.80		0.0073		0.0104		0.0034	

Note: Means with same letters are not significantly different

under control was 369.3g but under sprayed it was significantly reduced to 45.5g. Pairwise comparison of mean revealed highly significant differences in biomass in all the genotypes except ICCV 10. Prostko et al. (2009) studied the response of imidazolinone-resistant sunflower varieties to post-emergence application of imazapic. They reported that the herbicide had no significant effect on biomass, plant height and yield in some genotypes.Mean 100-seed weight across genotypes was 21.06g and 17.13g under control and sprayed condition respectively. Significant reduction in 100-seed weight was observed for ICCV 3, ICCV 03407, ICCV 10, ICCV 00305, Pusa 5028 and ICCL 82104 under sprayed condition. Under sprayed condition, ICCV 00305 had highest harvest index (HI) while ICCV 97107 had lowest. The HI increased under sprayed condition in genotypes ICCV3, ICCV 03407, ICCIL 04001, ICCV 10, ICCV 00305, ICCV 96003, ICC 1710 and ICCL 82104.

Effect of herbicide on branched chain amino acid content

The percent valine content differed significantly under sprayed and control conditions with mean 0.0544% and 0.0371% respectively (Fig. 4). Pairwise comparison

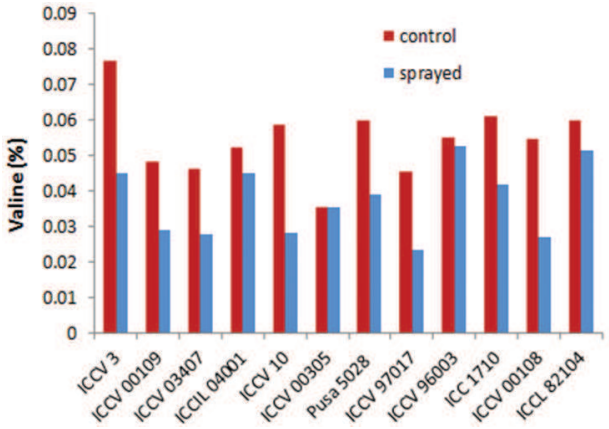

Fig. 4. Mean valine content under control and sprayed condition

of mean values of genotypes revealed non-significant difference in the genotypes ICCIL 04001, ICCV 00305, ICCV 96003 and ICCL 82104. The genotype ICCV 00305 showed no reduction in percent valine content in sample under sprayed condition while minimum reduction was observed in genotype ICCV 96003 (4%). Non-significant difference in the percent isoleucine content under control and sprayed conditions was recorded for all the genotypes except ICCV 3 and ICCV 96003 (Fig. 5). Some genotypes showed drastic

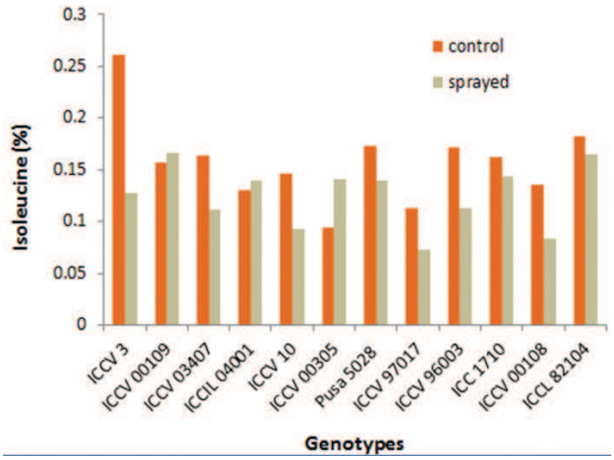

Fig. 5. Mean isoleucine content under control and sprayed conditions

increase (upto 51% in ICCV 00305) in isoleucine content of sample under sprayed conditions. Percent leucine content showed non-significant difference under control and sprayed for genotypes ICCV 03407, ICCIL 04001, ICCV 10, ICCV 96003, ICC 1710, ICCV 00108 and ICCL 82104 while the genotypes ICCV 3, ICCV 00109, ICCV 00305, Pusa 5028 and ICCV 97017 showed significant difference in percent leucine content of sample. Maximum reduction was found in Pusa 5028 (31%) (Fig. 6). While minimum reduction was

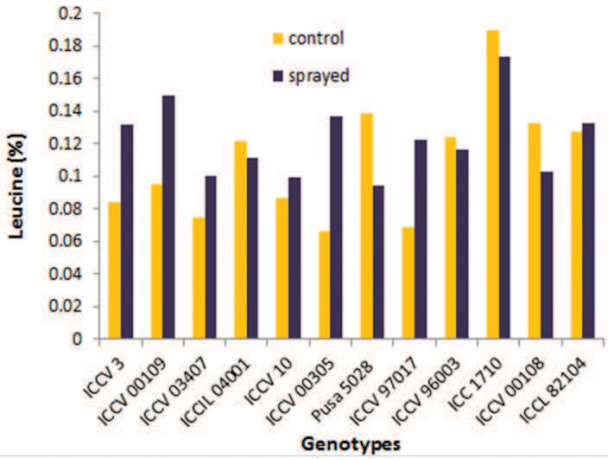

Fig. 6. Mean leucine content under control and sprayed conditions

observed in genotypes ICCV 96003 (6%) followed by ICCIL 04001 and ICC 1710 (8%). The genotype ICCV 00305 showed no reduction in percent valine content in sample.

Imazethapyr inhibits Aceto-hydroxy acid synthase (AHAS, E.C. 4.1.3.18) or Aceto-lactate Synthase (ALS) enzyme involved in the biosynthesis

Table 3. Tolerant and susceptible genotypes identified based on Tukey's LSD of mean

Trait	Tolerant	Susceptible
Yield	ICCL 82104, ICC 1710 and ICCV 10	ICCV 97017
NDVI	ICCV 96003	ICCL 82104
HI	ICCV 10, ICC 1710, ICCL 82104 and ICCV 96003	ICCV 97017
Valine	ICCV 96003, ICCL 82104, ICC 1710 and ICC 00305	ICCV 10, ICCV 97017
Isoleucine	ICCV 3, ICCV 96003, ICCL 82104 and ICC 00305	ICCV 97017, ICCV 00109
Leucine	ICCV 96003, ICC 1710, ICCV 10 and ICCL 82104	Pusa 5028, ICCV 97017

of Branched chain amino acids (BCAA) valine, isoleucine and leucine. This enzyme is the target site for many herbicides including all members of sulfonylurea and imidazolinone families (Duggleby et al. 2008). Significant decrease in BCAA content can be used as an indicator of AHAS inhibition due to herbicide. Muhitch et al. (1987) found that after treatment with imazapyr, there was decrease in extractable ALS in maize while the levels of other enzymes remained unchanged. Royuela et al. (2000) also estimated the BCAA content in pea in order to study growth inhibition by Imazethapyr. Research work on AHAS-inhibiting herbicides and their effect on BCAA synthesis have been reviewed by Zhou et al. (2007). Similar results have been reported by Ray (1984) in pea; Anderson et al. (1985) in maize and Shaner and Reider (1986) in maize, where they found that levels of valine, leucine and isoleucine (BCAA) decreased significantly in the herbicide treated plants. A recent study conducted on Imazethapyr toxicity in *Arabidopsis thaliana* suggested that Imazethapyr decreases the synthesis of BCAA through inhibition of activity of ALS enzyme without affecting the transcription or translation of ALS (Qian et al. 2015).The genotypes tolerant to post-emergence herbicide Imazethapyr identified based on non-significant reduction in the yield attributes and BCAA (valine, leucine and isoleucine) content in the sample were ICC 82104, ICCV 10, ICCV 96003, ICC 00305 and ICC 1710 while ICCV 97107 was most susceptible (Table 3). These tolerant genotypes can be used to study the genetics and mapping of gene(s) for herbicide tolerance in chickpea and in breeding programs for developing lines with tolerance to post-emergence herbicide Imazethapyr. Herbicide tolerant chickpea cultivars will bring down cost of cultivation making it more remunerative for the farmers.

Authors' contribution

Conceptualization of research (NRP, RKS, ST); Designing of the experiments (ST, PMG, NRP, RKS); Contribution of experimental materials (ST, PMG, CE, VSH); Execution of field/lab experiments and data collection (NRP, RKS, SKC, MKS); Analysis of data and interpretation (NRP, RKS, PKJ, ST); Preparation of manuscript (NRP, RKS, ST).

Declaration

The authors declare no conflict of interest.

Acknowledgement

The authors are thankful to Department of Agriculture, Cooperation and Farmers Welfare, Government of India for the financial support in the form of project and Maize Genetics Unit of Division of Genetics, Indian Council of Agricultural Research (ICAR) – IARI for UPLC analysis.

References

Al-Khatib K. and Miller J. F. 2000. Registration of four genetic stocks of sunflower resistant to imidazolinone herbicides. Crop Sci., **40**(3): 869-869.

Al-Khatib K., Baumgartner J. R., Peterson D. E. and Currie R. S. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci., **46**: 403-407.

Al Thahabi S. A., Yasin J. Z., Abu Irmaileh B. E., Haddad N. I. and 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. and Crop Sci., **172**(5): 333-341.

Anderson P. C. and Hibberd K. A. 1985. Evidence for the interaction of an imidazolinone herbicide with leucine, valine, and isoleucine metabolism.Weed Sci., **33**: 479-483.

Bhan V. M. and Kukula S. 1987. Weeds and their control in chickpea.The chickpea, 319-328.

Chaturvedi S. K., Muraleedhar A., Gaur P. M., Neelu M., Singh K. and Nadarajan N. 2014. Genetic variations for herbicide tolerance (Imazethapyr) in chickpea

(*Cicer arietinum*). Indian J. Agric. Sci., **84**(8): 968-970.

Duggleby R. G. and Pang S. S. 2000. Acetohydroxyacid synthase. BMB Reports, **33**(1): 1-36.

Gaur P. M., Jukanti A. K., Samineni S., Chaturvedi S. K., Singh S., Tripathi S. and Mishra N. 2013. Large genetic variability in chickpea for tolerance to herbicides imazethapyr and metribuzin. Agronomy, **3**(3): 524-536.

Hartwig E. E. 1987. Identification and Utilization of Variation in Herbicide Tolerance in Soybean (*Glycine max*) Breeding. Weed Sci., **35**(S1): 4-8.

FAOSTAT 2014. http://faostat.fao.org/faostat/. Accessed on 3 January 2017.

Kolkman J. M., Slabaugh M. B., Bruniard J. M., Berry S., Bushmanb B. S., Olungu C. and Leon A. 2004. Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. Theor. Appl. Geneti., **109**(6): 1147-1159.

Lee H., Rustgi S., Kumar N., Burke I., Yenish J. P., Gill K. S. and Ullrich S. E. 2011. Single nucleotide mutation in the barley acetohydroxy acid synthase (AHAS) gene confers resistance to imidazolinone herbicides. Proceedings of the National Academy of Sci., **108**(21): 8909-8913.

Malik S. R., Bakhsh A., Ahsan M. A., Iqbal U. and Iqbal S. M. 2009. Assessment of Genetic Variability and Interrelationship among some Agronomic Traits in Chickpea. International J. Agric. and Bio. **12**(1): 1814-9596.

Mergoum M., Singh P. K., Anderson J. A., Peña R. J., Singh R. P., Xu S. S. and Ransom J. K. 2009. Spring wheat breeding. In *Cereals* Springer, US. 127-156.

Mohammadi G., Javanshir A., Khooie F. R., Mohammadi S. A. and Zehtab Salmasi S. 2005. Critical period of weed interference in chickpea. Weed Res., **45**(1): 57-63.

Muhitch M. J., Shaner D. L. and Stidham M. A. 1987. Imidazolinones and Acetohydroxyacid Synthase from Higher Plants Properties of the Enzyme from Maize Suspension Culture Cells and Evidence for the Binding of Imazapyr to Acetohydroxyacid Synthase in vivo. Plant Physio., **83**(2): 451-456.

Ping Si. 2009. A possible herbicide option for chickpea: Metribuzin as post-emergent. Beanstalk, **10**(1).

Plew J. N., Hill G. D. and Dastgheib F. 1994. Weed control in chickpeas (*Cicer arietinum*). In Proceedings Annual Conference-Agronomy Society of New Zealand, **24**: 117-124.

Prostko E. P., Grey T. L. and Davis J. W. 2009. Imidazolinone-resistant sunflower tolerance to imazapic. Weed Technology, **23**(1): 188-190.

Qian H., Lu H., Ding H., Lavoie M., Li Y., Liu W. and Fu Z. 2015. Analyzing *Arabidopsis thaliana* root proteome provides insights into the molecular bases of enantioselective imazethapyr toxicity. Scientific Rep., **5:** 11975.

Ray T. B. 1984. Site of action of chlorsulfuron inhibition of valine and isoleucine biosynthesis in plants. Plant Physio., **75**(3): 827-831.

Royuela M., Gonzalez A., Gonzalez E. M., Arrese-Igor C., Aparicio-Tejo P. M. and Gonzalez-Murua C. 2000. Physiological consequences of continuous, sublethal imazethapyr supply to pea plants. J. Plant Physio., **157**(3): 345-354.

Shaner D. L. and Reider M. L. 1986. Physiological responses of corn (*Zea mays*) to AC 243,997 in combination with valine, leucine, and isoleucine. Pesticide Biochem. and Physio., **25**(2): 248-257.

Shaner D. L., Anderson P. C. and Stidham M. A. 1984. Imidazolinones potent inhibitors of acetohydroxyacid synthase. Plant Physio., **76**(2): 545-546.

Si P., Quealy J., Sweetingham M. and Buirchell B. 2008. Improved herbicide tolerance in narrow-leafed lupin (*Lupinus angustifolius* L.) through utilizing natural variation and induced mutants. "In Lupins for Health and Wealth - Proceedings of the 12th International Lupin Conference" (Palta J. A. and Berger J. B. eds). 14-18 Sep. 2008, Fremantle, Western Australia. International Lupin Asso., Canterbury, New Zealand. ISBN 0-86476-153-8.

Solh M. B. and Pala M. 1990. Weed control in chickpeas. In Present status and future prospects of chickpea crop production and improvement in the Mediterranean countries (eds. Saxena M. C., Cubero J. I. and Wery J.) Zaragosa: CIHEAM: 93-99.

Srivastava P., Anjana P. and Sinha D. P. 2011. Genetic diversity analysis in different varieties of black gram using RAPD markers. J. of Plant Breeding and Crop Sci., **3**(3): 53-59.

Taran B., Holm F. and Banniza S. 2013. Response of chickpea cultivars to pre-and post-emergence herbicide applications. Canadian J. Plant Sci., **93**(2): 279-286.

Taran B., Warkentin T. D., Vandenberg A. and Holm F. A. 2010. Variation in chickpea germplasm for tolerance to imazethapyr and imazamox herbicides. Canadian J. Plant Sci., **90**(1): 139-142.

Thompson C. and Taran B. 2014. Genetic characterization of the acetohydroxyacid synthase (AHAS) gene responsible for resistance to imidazolinone in chickpea (*Cicer arietinum* L.). Doctoral dissertation, **127**(7): 1583-1591.

Walter K. L., Strachan S. D., Ferry N. M., Albert H. H., Castle L. A. and Sebastian S. A. 2014. Molecular and phenotypic characterization of Als1 and Als2 mutations conferring tolerance to acetolactate synthase herbicides in soybean. Pest Management Sci., **70**(12): 1831-1839.

Zhou Q., Liu W., Zhang Y. and Liu K. K. 2007. Action mechanisms of acetolactate synthase-inhibiting herbicides. Pesticide Biochem. and Physio., **89**(2): 89-96.