



Genetic variations for post emergence herbicide tolerance in field pea (*Pisum sativum*)

A K PARIHAR^{1*}, N KUMAR², C P NATH³, A K SINGH⁴, S K CHATURVEDI⁵, G P DIXIT⁶ and N P SINGH⁷

ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208 024, India

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ABSTRACT

Weeds are highly competitive to the crops for nutrient and water from soil, sunlight, space and also harbour many insect-pest and diseases, consequently lead to drastic yield reduction. *Pisum sativum* is sensitive to the most of the potential post emergence herbicides and thus, effective weed management is a difficult assignment particularly the later flush of weed emergence. The identification of herbicide resistant genotypes is the potential way to develop herbicide tolerant varieties as well as to control weeds and minimise yield losses. So far no report is available for herbicide tolerant genotypes in field pea. Therefore, an attempt has been made to identify the genotypes as a source of resistance to the post-emergence herbicide metribuzin. Total 822 genotypes were examined for their sensitivity under preliminary screening against metribuzin at 0.5 kg ai/ha during the winter season of 2015-16. Of the tested genotypes, a set of 85 promising genotypes were re-evaluated with same dose during the winter season of 2016-17 with visual phyto-toxicity score. The results of experiment revealed that there was a huge amount of genetic variation for tolerance against metribuzin in field pea. The frequency distribution grouped the genotypes as tolerant (1), moderately tolerant (5), susceptible (18), and highly susceptible (61) categories. None of the genotypes showed highly tolerant reaction. Notably, accession P-637 witnessed tolerance and other five accessions, viz. P-729, P-647, P-1075, P-2016, and P-1448-2 registered moderately tolerance reaction against metribuzin. Hence, aforesaid promising genotypes may be utilized as donor to speed up breeding for development of herbicide tolerant varieties in field pea and in other genetical studies too.

Key words: Herbicide tolerance, Metribuzin, Phyto-toxicity, *Pisum sativum*, Post-emergence herbicide

Dry pea or field pea (*Pisum sativum* L.) is an important cool season legume crop of the world and is a rich source of protein (21.1-32.9%), carbohydrate, minerals and vitamins which are good for human and livestock consumption and health (Parihar *et al.* 2016). The productivity of this crop is affected by several biotic and abiotic factors. Among these factors, weeds can cause > 75% reduction in crop yield (Tripathi *et al.* 2001, Singh *et al.* 2016). Weeds have competition to the crops for nutrient, water, sunlight, space and also harbour many insect-pest and diseases (Gaur *et al.* 2013), subsequently, the performance of crop in term of the yield is drastically reduced. Presently, pre-emergence herbicide (pendimethalin) and manual weeding at 30-

35 days after sowing is recommended in pea. However, manual weeding is proving difficult because of labour scarcity at critical time of weeding and increasing cost (Chaturvedi *et al.* 2014). Therefore, to control the later flush of weeds, use of post-emergence herbicides becomes important. But, no post-emergence herbicide is available for controlling broad-leaved weeds like *Medicago denticulata*, *Vicia sativa*, *Convolvulus arvensis*, *Chenopodium album*, *Phalaris minor* and others. Therefore, an effective and efficient weed management is essential to achieve potential yield (Nath *et al.* 2017). Pea is a poor competitor to weeds owing to its slow initial growth and wider plant spacing that provide congenial environment for weeds to grow. Besides, its succulent plant type also do not allow second manual weeding as it can cause damage to the crop. Therefore, herbicide tolerance cultivars may offer larger elasticity for use of post-emergence herbicides and are immediately required by the *P. sativum* growing farmers. Quizalofop-p-ethyl, clodinafop-propagyl, imazethapyr, and imazethapyr + imazamox (ready-mix) are post-emergence herbicides

¹Scientist, Crop Improvement, ²Principal Scientist, Crop Production, ³Scientist, Crop Production, ⁴Senior Research Fellow, ⁵Principal Scientist, Crop Improvement, ⁶Project Coordinator, AICRP on Chickpea; ⁷Director, ICAR-Indian Institute of Pulses Research, Kanpur 208 024.

used in many rainy season legume crops. Metribuzin (4-Amino-6-tert-butyl-3-methylsufanyl-1, 2, 4-triazin-5-one) is a potential broad-spectrum herbicide used in soybean and wheat crops. It inhibits the photosystem-II pathway, which demonstrates adverse effect on the fully developed leaves and subsequently on plant growth. So far no report is available on herbicide tolerance in *P. sativum*. Also, till date no systematic study was conducted to see the efficacy of this post-emergence herbicide in fieldpea. It is well established that genotypes/cultivars resistance to herbicide is the most potential way to minimize losses due to weeds. Thus, the present investigation was attempted to identify the sources of resistance to the post-emergence herbicide metribuzin in *P. sativum*.

MATERIALS AND METHODS

Preliminary screening

Total 822 peas genotypes including germplasm accessions (indigenous and exotic collections) and released cultivars/advanced breeding lines were used for preliminary screening during winter season of the 2015-16 against post-emergence herbicide metribuzin at 0.5 kg ai/ha in an Augmented Design at Research Institute, Kanpur, Uttar Pradesh, India (Fig 1). The plants were grown on 1-m long rows with 0.3 m spacing between rows and 0.1 m between plants. Metribuzin at 0.5 kg ai/ha was applied 25 days after sowing through manually operated knapsack sprayer fitted

with flat fan nozzle (pressure: 200 kPa) using 400 litre of water/ha during cool hours of the day when there was little or no wind for uniform spray and absorption. The plants were scored for herbicide tolerance at 15 days after herbicide application (DAHA) on a 1–5 scale (Gaur *et al.* 2013). No weeding was done prior to or post herbicidal application. The visual observations were recorded for toxicity and its effect on upper parts of plant i.e. leaves and stem for every individual genotype.

Second year screening

A set of diverse 85 promising genotypes selected on the basis of first year of screening were evaluated along with control (same set of genotypes without spray) to validate the herbicide (metribuzin 0.5 kg ai/ha) tolerance reaction observed in the preliminary experiment. The crop geometry, package and practices, herbicide dose, herbicide application and scoring methods were adopted similar to the preliminary screening. Additionally, another set of same genotypes was also raised as control i.e. without herbicide application, to observe the difference within genotype after spray and without spray (Fig 1). The plants were scored for herbicide toxicity on three different stages, i.e. 15 days after herbicide application (DAHA) spray (DAHA), 30 DAHA and 60 DAHA.

The analysis of variance was performed using SAS 9.2 software (SAS Institute). The original data were rescaled by multiplying with 1000 before analysis to get

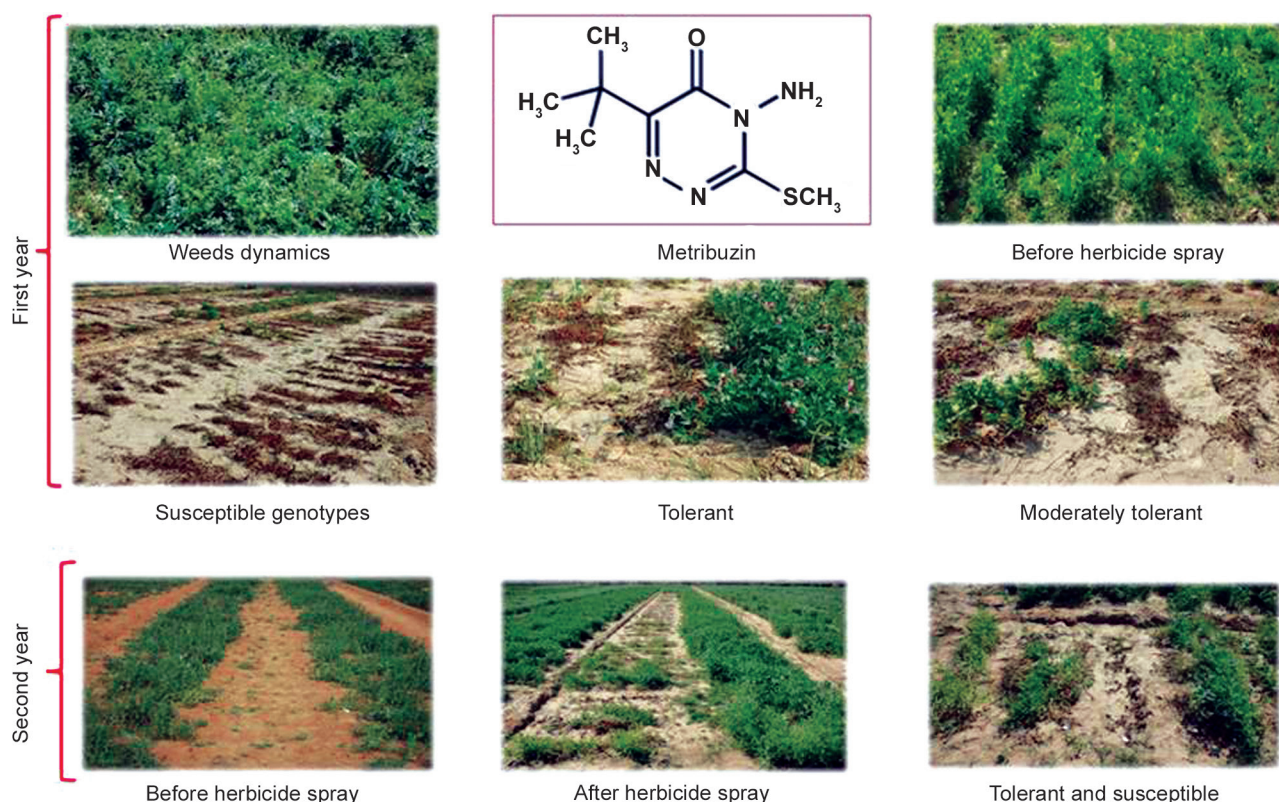


Fig 1 Field view of fieldpea crop with weed dynamics and crop behaviour before and after spray herbicide spray during winter 2015-16 (first year) and 2016-17 (second year).

Table 1 Toxicity scores against post emergence herbicide (metribuzin 0.5 kg ai/ha) in fieldpea during winter season of 2016-17

Genotype	15 DAHA	30 DAHA	60 DAHA	Genotype	15 DAHA	30 DAHA	60 DAHA
P-729	3.0	2.0	3.0	P-1358	4.0	5.0	5.0
P-647	2.0	3.0	3.0	P-781	4.0	4.0	5.0
P-1075	3.0	2.0	3.0	P-1807	5.0	5.0	5.0
P-1573	4.0	4.0	5.0	P-1034	3.0	3.0	5.0
HUDP-15	4.0	5.0	5.0	P-1443-1	3.0	4.0	5.0
P-637	2.0	2.0	2.0	P-471	3.0	4.0	5.0
P-1297-35-1	3.0	3.0	4.0	P-639	5.0	5.0	5.0
P-2016	3.0	2.0	3.0	P-1375	4.0	5.0	5.0
P-706	4.0	4.0	4.0	P-1448-2	2.0	3.0	3.0
P-700	4.0	4.0	4.0	P-1001	5.0	5.0	5.0
P-705	4.0	4.0	4.0	P-6586	5.0	5.0	5.0
P-999	4.0	4.0	4.0	P-1297-6-1	3.0	3.0	4.0
P-1042	4.0	3.0	4.0	P-1297	5.0	5.0	5.0
P-1046	4.0	4.0	4.0	P-3	4.0	4.0	4.0
P-1070	4.0	4.0	4.0	P-1430-2	4.0	4.0	5.0
P-1176-1	5.0	5.0	5.0	P-600	4.0	4.0	4.0
P-1301	3.0	2.0	4.0	P-1295	4.0	4.0	5.0
P-1384-1	5.0	5.0	5.0	P-1	5.0	5.0	5.0
P-1805	4.0	4.0	5.0	P-782	4.0	5.0	5.0
IVD-99-9	4.0	4.0	4.0	P-744	4.0	4.0	5.0
EC-382476	4.0	4.0	5.0	P-1808	5.0	5.0	5.0
ET-5117	2.0	3.0	4.0	P-841	5.0	5.0	5.0
ET-5122	4.0	4.0	5.0	P-5	5.0	5.0	5.0
P-1545-1	4.0	4.0	5.0	P-1297-22	4.0	4.0	4.0
P-201	4.0	4.0	5.0	P-815	4.0	5.0	4.0
P-1297-27-1	5.0	5.0	5.0	P-705	4.0	5.0	5.0
P-1436-5	5.0	5.0	5.0	IP2K 76	3.0	4.0	5.0
IPFD 99-13	4.0	4.0	5.0	IP2K107	3.0	3.0	5.0
P-1541-33	5.0	4.0	5.0	IP2K79	4.0	5.0	5.0
P-1544-4	4.0	4.0	5.0	IP2K 77	4.0	5.0	5.0
P-1545-2	5.0	5.0	5.0	IP2K 119	3.0	4.0	5.0
P-1456-A-3	5.0	5.0	5.0	IVD 99-6	5.0	5.0	5.0
P-1457-1	5.0	5.0	5.0	IVD-99-11	4.0	5.0	5.0
P-1547-2	4.0	4.0	5.0	KSP-9	4.0	4.0	5.0
P-1601	5.0	5.0	5.0	EC 329577	5.0	5.0	5.0
P-1604	5.0	5.0	5.0	EC 389377	4.0	4.0	5.0
P-1622	5.0	5.0	5.0	EC 329568	4.0	4.0	4.0
P-1621	5.0	5.0	5.0	EC 329576	5.0	5.0	5.0
P-91-3	5.0	5.0	5.0	ET 5106	4.0	5.0	5.0
P-107	4.0	4.0	5.0	ET 45190	4.0	4.0	5.0
P-107-12	4.0	4.0	5.0	P-867	5.0	5.0	5.0
P-122-11	5.0	5.0	5.0	IPFD 1-10	4.0	4.0	4.0
P-122-12	4.0	4.0	5.0				

better presentation of source variance. In addition, other graphical representations were made using Microsoft excel worksheet 2007.

RESULTS AND DISCUSSION

Total 822 field pea genotypes were screened against post-emergence herbicide metribuzin for post emergence herbicide tolerance. The analysis of variance revealed that ample amount of genetic variation exists for tolerance against metribuzin at 0.5 kg ai/ha. During the preliminary screening (2015-16), large amount of variability was observed on the basis of visual observations and toxicity (Fig 1 and 2) and promising genotypes were extracted for further confirmation. Similarly, in the 2nd year (2016-17) of screening, 85 promising genotypes also witnessed huge variability for tolerance against metribuzin (Fig 3 & Table 1). These findings are in accordance with earlier reports

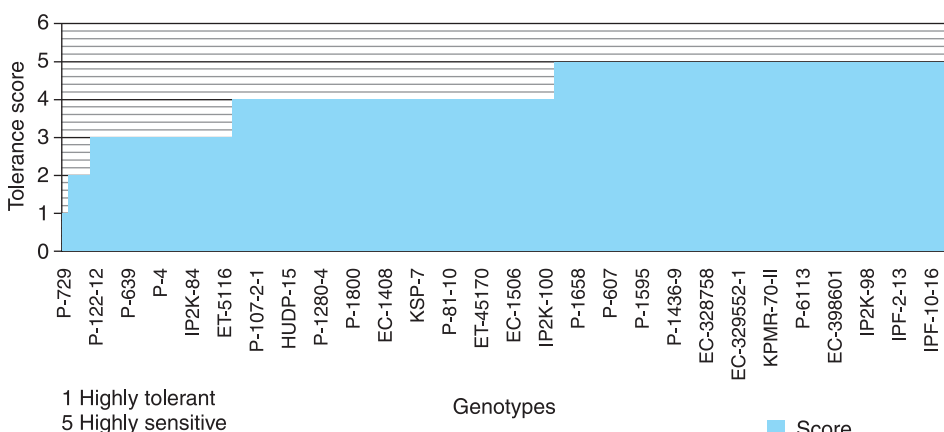


Fig 2 Magnitude of variation in fieldpea genotypes (822) for tolerance to post emergence herbicide Metribuzin during winter 2015-16.

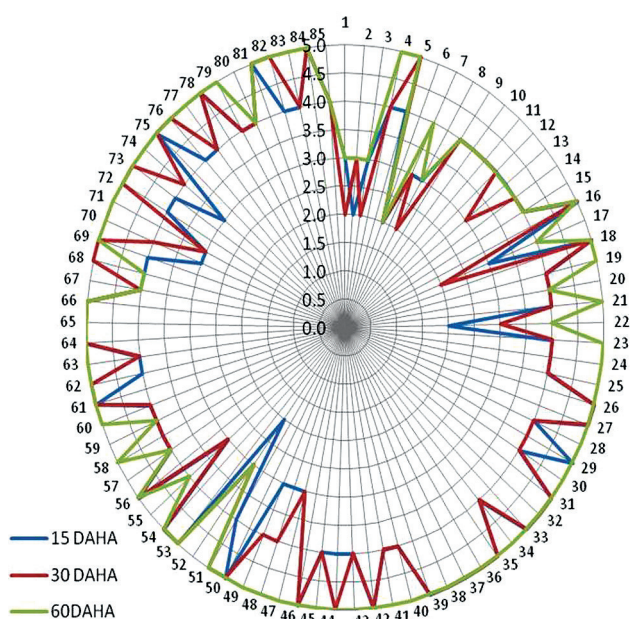


Fig 3 Spectrum of post-emergence herbicide (metribuzin 0.5 kg ai/ha) toxicity in 85 genotypes of fieldpea during winter season of 2016-17 at 15 days after herbicide application spray (DAHA), 30 DAHA and 60 DAHA.

on chickpea and peas (Gaur *et al.* 2013, Chaturvedi *et al.* 2014, Hanson and Thill 2001, Nath *et al.* 2017). Because of photosystem-II inhibition, metribuzin exhibited adverse effect on the fully developed leaves. In term of phyto-toxicity symptoms, metribuzin caused complete burning/death of the plants in highly susceptible genotypes with 100% mortality (phyto-toxicity score 5). Further, tolerant lines had healthy plant appearance with no leaf burning/chlorosis (Fig 1). After application of herbicide, few genotypes recovered with secondary growth at 30-35 days after herbicide application leading to flowering and pod set. This could be due the phenological plasticity of the pea genotypes. The susceptible and highly susceptible lines exhibited higher magnitude of leaf burning (phyto-toxicity score >3) within 7 days of herbicide application (Ramakrishan *et al.* 1992, Gaur *et al.* 2013).

The response of individual genotypes for post-emergence

metribuzin application varied during the scoring period. The frequency distribution (Fig. 4) clearly witnessed the periodical changes in the performance of genotypes for metribuzin tolerance. At the initial stage (i.e. 15 days after herbicide application) 4, 12, 42, and 27 genotypes were scored as tolerant, moderately tolerant, susceptible and highly susceptible categories, respectively. After 30 DAHA, only 5, 8, 36 and 36 genotypes harboured in tolerant, moderately tolerant,

susceptible and highly susceptible group, respectively. Similarly, at 60 DAHA, 1, 5, 18, and 61 genotypes were grouped as tolerant, moderately tolerant, susceptible, and highly susceptible, respectively. The periodical variations in the genotypes were due to the residual activity of metribuzin

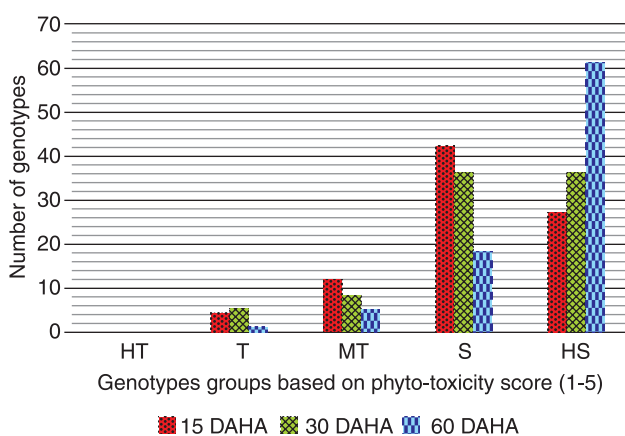


Fig 4 Frequency distribution of 85 genotypes of fieldpea for post-emergence herbicide (metribuzin 0.5 kg ai/ha) toxicity during winter season of 2016-17 at 15 days after herbicide application spray (DAHA), 30 DAHA and 60 DAHA.

at 0.5 kg ai/ha. For instance, the highly susceptible genotypes showed the phyto-toxicity immediately after application of herbicides. While at later stages, metribuzin caused higher phyto-toxicity to some genotypes, whereas, few genotypes recovered over time. None of the genotypes reacted as highly tolerant at any stage of scoring in the crop. The behaviour of some genotypes fluctuated for metribuzin toxicity over the scoring period. At 30 DAHA, the reactions of genotypes shifted from tolerance to moderately tolerance category, moderately tolerance into susceptible category and susceptible to highly susceptible category. The shift in performance may be due to the moisture scarcity in soil which resulted to the reduced efficacy of post-emergence herbicide and subsequently, change in the genotype performance was noticed. The efficacy of post-emergence herbicide reduced under water or other stresses as plants had smaller leaves, thicker cuticles and more deposited wax layer under soil moisture stress as compared to plants grown under sufficient moisture availability. In addition, plants gradually closed their stomata due to water stress, leading to a decline in the photosynthesis and other biosynthetic process (Hull 1970, Turner and Begg 1981, Kudsk and Kristensen 1992) resulting into reduced translocation efficiency. Such changes in internal and external structure of leaves might influence the herbicide metabolism (Bouchard *et al.* 1982, Runyan *et al.* 1982). However, visible change was observed in terms of genotypic response to herbicide toxicity after irrigation. Genotypes ET 5117 and P-1448-2 which expressed tolerance to metribuzin reverted to susceptible or moderately group after irrigation. Earlier studies also reported that killing of weeds or plants is easier with post-emergence herbicides when they are in good and healthy conditions. Therefore, many biosynthetic processes might have initiated in the plant after irrigation and translocated the herbicide to the point of action which increased or decimated the herbicide toxicity in the plants. It was observed that the appearance of genotypes before irrigation was pretty good and they were trying to recover as their growth was either checked or it was very slow due to water stress and all the physiological process occurred were reduced. Therefore, the herbicide could not be metabolized to other parts of plant where it may be develop toxicity symptoms. In other words, the metribuzin which persisted in soil for prolonged period, got activated after irrigation and caused toxicity. Therefore, the herbicide got metabolized to other parts of plant where it might develop toxicity symptoms. The genotypes having higher inherent tolerance showed lower phyto-toxicity in pea and vice-versa. These finding are corroborating to the earlier results (Conn and Cameron 1988, Shaw 1985, Sager 1977, Anonymous 2007, Datta *et al.* 2009). Importantly, genotype P-647 (phyto-toxicity score 2) has consistently demonstrated tolerance for herbicide toxicity during the scoring period (stressed and unstressed condition). The consistent tolerance expressed by aforementioned genotype indicates that this may have the ability of quick conversion of toxic effect of metribuzin into non-toxic metabolites. These finding are in accordance to earlier reports (Gillespie *et al.* 2011,

Anonymous 2007).

Based on the overall scoring, accession P-637 witnessed tolerance (phyto-toxicity score 2) and other five accessions, viz. P-729, P-647, P-1075, P-2016 and P-1448-2 registered moderately tolerance reaction (phyto-toxicity score 3) against metribuzin. Similar information of metribuzin tolerance has also been reported in lupin and chickpea (Si *et al.* 2006, 2010, Gaur *et al.* 2013). In legume crops, several herbicides including metribuzin have been recommended for weed management in Australia, Turkey, Canada (Datta *et al.* 2009). Presently, metribuzin is not recommended anywhere for *P. sativum* owing to its sensitivity to this herbicide. Therefore, herbicide tolerant cultivars of *P. sativum* are urgently needed to provide a better option for weed control over hand weeding and for broad-spectrum control of weeds through post-emergence herbicides including metribuzin. In addition, management of weeds through the herbicides is very much needed in the developing countries like India for reducing the cost of cultivation and to make *P. sativum* cultivation more remunerative.

Finally, the herbicide tolerant and moderately tolerant genotypes identified in present study would be useful in the development of herbicide tolerant cultivars. Furthermore, it would also be useful in generating basic information on inheritance, mapping and tagging of genes for herbicide (metribuzin) tolerance in *Pisum sativum*.

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