

Evaluation of germination and seedling tolerance index of black gram genotypes in response to herbicide glyphosate

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Abstract: Herbicides are expected to protect crop plants from weed competition without harming the crop plants. Glyphosate (N-[Phosphonomethyl] glycine) is a non selective systemic herbicide used for control of annual and perennial plants including grasses, sedges, broad leaved weeds and woody plants. But broad spectrum herbicides like glyphosate can also damage non target plants. Black gram is one such target crop and therefore it becomes necessary to select ideal blackgram genotypes, which may be tolerant to herbicide stress and produce substantial yield under stress conditions. Herbicide tolerance of six black gram genotypes was investigated at the germination and seedling growth stages. Seed germination percentage, survival percentage, seedling root length, seedling fresh and dry weight, seedling vigour index and tolerance index was assessed after 7 and 15 days of herbicide treatment. Results showed that increasing glyphosate concentrations caused reduction in all the studied parameters of seedlings in all the genotypes but response was different among genotypes. Root length inhibition was more pronounced than shoot length inhibition and thus can also be chosen as selection criteria for screening of tolerant and susceptible genotypes. Decrease in root length was maximum in Azad-2 and PU-31 than the other four genotypes indicating their lowest tolerance to glyphosate. Tolerance index was highest for PU-19, IPU-94-1 and lowest for PU-31 and Azad-2. More vigorous cultivars like PU-19, Azad-2 and IPU-94-1 could be considered as plant materials useful for future development of herbicide tolerant cultivars in plant breeding.

Keywords: Black gram genotypes, genotypic variation, glyphosate, herbicide tolerance, tolerance index, vigour index.

Introduction

Herbicide is a chemical substance used to destroy or inhibit the growth of plants, especially weeds. Glyphosate (N-[Phosphonomethyl] glycine) is a post-emergent, systemic and non-selective (or broad-spectrum) herbicide that controls most annual and perennial weeds (1). Glyphosate is non-selective because it is unable to distinguish between crops and weeds (2). It is mainly absorbed into the plant through the leaves and then transported throughout the plant where it acts on the plant's enzyme system. Glyphosate inhibits amino acid metabolism known as the shikimic acid pathway, its main target being enzyme 5-Enol-pyruvyl-shikimate-3-phosphate (EPSP) synthase (3). Black gram (*Vigna mungo* (L.) Hepper) is an important legume crop cultivated worldwide in tropical and subtropical regions of the world and is valued for high protein in its seeds. India is the largest producer and consumer of black gram in the world. Existence of genotypic variability for stress tolerance was reported by many workers in pulse crops viz. green gram (4, 5), cowpea (6), black gram and green gram (7, 8) and faba bean (12). In the present investigation, six black gram genotypes were screened for herbicide tolerance using Petri-dish method. Percent germination, percent

survival, root length, shoot length, fresh weight and dry weight were compared to see overall performance of different genotypes under study.

Materials and Methods

The investigation was carried out in the *in vitro* Culture and Plant Genetics unit in the Department of Botany, University of Lucknow, Lucknow. The experiment was arranged in three replicates in completely randomized design (CRD) and repeated twice. The seeds were procured from Seed Development Corporation, Lucknow. Healthy and uniform seeds were surface sterilized by using Tween-80 and washed with double distilled water five to six times. Various concentrations of Glyphosate (Round up)-1mM, 2mM, 4mM, 6mM, 8mM and 10mM were prepared and seeds of six genotypes-PU-19, PU-35, IPU-94-1, PU-31, Azad-1 and Azad-2 were treated by soaking for 24 hours. Water treated seeds were used as control. Twenty seeds from each treatment were inoculated on moist blotting paper in 9 cm. petri- dishes. Readings were taken after 7 and 15 days after treatment (DAT). In order to avoid infection, the blotting paper was changed every 2 days. Dishes were inspected daily and sterilized water added as required.

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Seeds were considered germinated with the emergence of radicle. Number of seeds germinated and length of seedlings were observed and measured in 7 day seedlings. Root lengths were measured in cm/plant and fresh weights of seedlings were taken by using an electrical single pan balance. The dry weight was taken after drying the seedlings in a hot air oven at 60° C till the weight stabilized.

Growth measurement:

1. **Germination Percentage:** It was calculated according to the germination count taken after 7 days for 20 seeds per replicate and expressed as percentage according to following equation (9)

$$\text{Germination Percentage} = \frac{\text{Number of germinated seeds 7 DAT}}{\text{Total number of seeds}} \times 100$$

2. **Survival Percentage:** It was calculated by following formula

$$\text{Survival Percentage} = \frac{\text{Number of surviving seedlings 15 DAT}}{\text{Total number of seeds}} \times 100$$

3. **Seedling Vigour Index:** It was calculated according to formula following (10).

Vigour index = Germination percentage X Length of seedlings

4. **Tolerance Index:** It was calculated according to following equation (11).

$$\text{Tolerance index} = \frac{\text{Length of longest root in treatment}}{\text{Length of longest root in control}}$$

5. **Percent Reduction:**

$$\text{Percent reduction} = \frac{\text{Value in control} - \text{Value in treatment}}{\text{Value in control}} \times 100$$

Statistical analysis of data

All obtained data were statistically analyzed. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5 and 1% level of probability using statistical software SPSS ver. 19.0 and twoway Anova was performed by Graphpad Prism ver. 5.0.

Results

Germination Percentage:

Mean values for germination percentage of six black gram genotypes differed significantly after herbicide stress induced by glyphosate treatment. Treatment with glyphosate led to a decrease in germination percentage in all the varieties.

Glyphosate not only reduced the germination percentage but also delayed the germination initiation in all black gram genotypes. In control maximum germination was in Azad-1 and IPU-94-1 (97.78±2.22) and minimum in PU-31 and Azad-2 (91.11±2.22). Glyphosate caused maximum percent decrease over control in germination percentage at 10mM of PU-35 and Azad-2 where germination was reduced up to 92.68% while minimum percent inhibition over control was found in PU-19 and Azad-1 (86.36%). Among treatment there was not significant difference at 1mM but significant ($P < 0.05$) reduction was found in germination percentage at 2, 4, 6, 8 and 10mM except PU-19 (Fig-1A). Two way anova showed a highly significant effect of genotype (factor 1) and concentration (factor 2) as well as interaction between two factors. Factor 1- $F_{5,84} = 8.713, p < 0.0001$; Factor 2- $F_{6,84} = 461.3, p < 0.0001$; factor 1x2- $F_{30,84} = 3.187, p < 0.0001$.

Survival percentage:

The seedling survival showed a marked difference in all the six genotypes. The percentage survival showed a gradual decrease with increasing glyphosate concentrations in all the six genotypes (Fig-1B). Significant ($p < 0.05$) reductions in seedling survival were observed from 2mM glyphosate concentration while in PU-35, IPU-94-1 and Azad-2 significant decrease was also found in 1mM. The percentage survival of seedlings at the highest glyphosate concentration (10mM) was 8.89±2.22 in PU-19, 4.44±2.22 in PU-35, 6.67±3.85 in IPU-94-1 and 6.67±0.00 in Azad-1 whereas it was only 2.22±2.22 in Azad-2 and PU-31. Maximum survival was obtained in control with the highest value in PU-19 (88.89±4.44) and lowest in Azad-2 (71.11±2.22). Percent reduction with respect to control in survival percentage at 10mM was PU-19 (90.00), PU-35 (94.44), IPU-94-1 (91.89), PU-31 (97.06), Azad-1 (91.43) and Azad-2 (96.88). Two way anova revealed a significant effect of genotype (factor -1) and concentration (factor -2) but not of interaction between two factors (genotype and concentration). Factor 1- $F_{5,84} = 9.741, p < 0.0001$; Factor 2- $F_{6,84} = 215.1, p < 0.0001$; factor 1x2- $F_{30,84} = 1.114, p < 0.34$.

Seedling Root Length:

This parameter is most sensitive for herbicide among all the parameters studied showing significant ($p < 0.05$) reduction in root length even at 1mM in all the genotypes. With

increasing concentration there was progressive decline in root length in all the genotypes. Highest root length (cm.) was observed in control and seedling root length in various genotypes was: PU-19(6.06 ± 0.27), PU-35 (5.77 ± 0.22), IPU-94-1(5.87 ± 0.18), PU-31(5.90 ± 0.21), Azad-1(5.83 ± 0.09) and Azad-2(5.23 ± 0.12) while at 10mM PU-19 and Azad-2 had highest and lowest root length (0.49 ± 0.03 and 0.23 ± 0.12) respectively. In concentrations higher than 2mM roots were stunted and there was decrease in root hair. Percent reduction with respect to control of root length increased with increase in glyphosate concentration in all the genotypes. Percent reduction with respect to control at highest dose 10mM was PU-19(91.85), PU-35 (93.99), IPU-94-1(90.91), PU-31(94.35), Azad-1(91.83) and Azad-2(95.14) (Fig-1C). This clearly indicates the relative tolerance among genotypes and we can conclude that the genotypes with less inhibition at highest glyphosate concentration (10mM) can more tolerate the herbicide stress. Two way anova for root length showed significant difference of genotype (factor 1) and concentration (factor 2) as well as interaction between genotype and concentration. Factor 1- $F_{5,84}=2.583$, $p<0.318$; Factor 2- $F_{6,84}=789.3$, $p<0.0001$; factor 1x2- $F_{30,84}=3.325$, $p<0.0001$. Thus it is cleared that black gram genotypes respond differentially to different herbicide concentration due to extremely significant interaction ($p<0.05$) between these two factors.

Seedling fresh weight:

Glyphosate treatment reduces fresh weight of plants. A reduction in fresh weight of seedlings was also observed during present study. Significant ($P<0.05$) differences was observed in seedling fresh weight between herbicide concentration and genotypes. The fresh weight of germinated seedlings of *V. mungo* genotypes was taken and it was found to decrease after the treatment in all the six genotypes. The fresh weight (g) of control seedlings was PU-19(1.15 ± 0.18), PU-35 (0.91 ± 0.03), IPU-94-1(1.23 ± 0.09), PU-31(0.85 ± 0.03), Azad-1(0.95 ± 0.03) and Azad-2(0.83 ± 0.05). At the highest glyphosate concentration fresh weight was 0.28 ± 0.04 in PU-19, 0.30 ± 0.03 in PU-35, 0.33 ± 0.01 in IPU-94-1, 0.23 ± 0.02 in PU-31, 0.30 ± 0.03 in Azad-1 and 0.24 ± 0.02 in Azad-2 (Fig-1D). Maximum and minimum reduction of fresh weight at 10mM was observed in PU-31(73.44%) and PU-35 (66.91%). Dose

dependent decrease was observed in fresh weight on increasing glyphosate concentration in all varieties. Two way anova for fresh weight of seedlings also revealed extremely significant difference of genotype (factor 1) and concentration (factor 2) as well as interaction between genotype and concentration. Factor 1- $F_{5,84}=35.55$, $p<0.0001$; Factor 2- $F_{6,84}=418.2$, $p<0.0001$; factor 1x2- $F_{30,84}=5.125$, $p<0.0001$.

Seedling Dry Weight:

Glyphosate also causes reduction in dry weight of seedlings in all genotypes. The dry weight of germinated seedlings of *V. mungo* genotypes was taken and it was also found to decrease after the treatment in all the six genotypes. The dry weight (g) of control seedlings was PU-19(0.33 ± 0.02), PU-35 (0.27 ± 0.02), IPU-94-1(0.28 ± 0.01), PU-31(0.27 ± 0.00), Azad-1(0.25 ± 0.02) and Azad-2(0.20 ± 0.01). At the highest glyphosate concentration 10mM dry weight was 0.06 ± 0.02 in PU-19, 0.06 ± 0.01 in PU-35, 0.05 ± 0 in IPU-94-1, 0.02 ± 0.03 in PU-31, 0.04 ± 0 in Azad-1 and 0.03 ± 0.02 in Azad-2 (Fig-1E). Significant differences in dry weight were observed for the treatments in all the genotypes. Maximum and minimum reduction of dry weight at 10mM was observed in PU-31(90.24%) and PU-35 (75.61%). Dose dependent decrease was observed in dry weight on increasing glyphosate concentration in all varieties. Two way anova for dry weight of seedlings showed extremely significant difference of genotype (factor 1) and concentration (factor 2) as well as interaction between genotype and concentration. Factor 1- $F_{5,84}=11.13$, $p<0.0001$; Factor 2- $F_{6,84}=257.3$, $p<0.0001$; factor 1x2- $F_{30,84}=2.823$, $p<0.0001$.

Vigour Index:

Vigour index of seedlings decreased with increasing concentration of herbicide in all the genotypes. The maximum vigour index (PU-19, 1216.67), (PU-35, 905.56), (IPU-94-1, 1284.22), (PU-31, 964.44), (Azad-1, 1231.56) and (Azad-2, 1184.44) were observed for their respective controls at 15 DAT. The minimum vigour index (PU-19, 12.22), (PU-35, 4.22), (IPU-94-1, 8.67), (PU-31, 3.11), (Azad-1, 10.44) and (Azad-2, 5.11) were observed in 10mM concentration (Fig-1F).

Tolerance Index:

The maximum tolerance index was observed in 1mM (PU-19, 0.88), (PU-35, 0.63), (IPU-94-1, 0.74), (PU-31, 0.72), (Azad-1, 0.81) and (Azad-2, 0.70) while minimum tolerance index was observed in

10mM (PU-19, 0.08), (PU-35, 0.06), (IPU-94-1, 0.09), (PU-31, 0.05), (Azad-1, 0.08) and (Azad-2, 0.05). Tolerance index decreased with increasing concentration of glyphosate (Fig-1G).

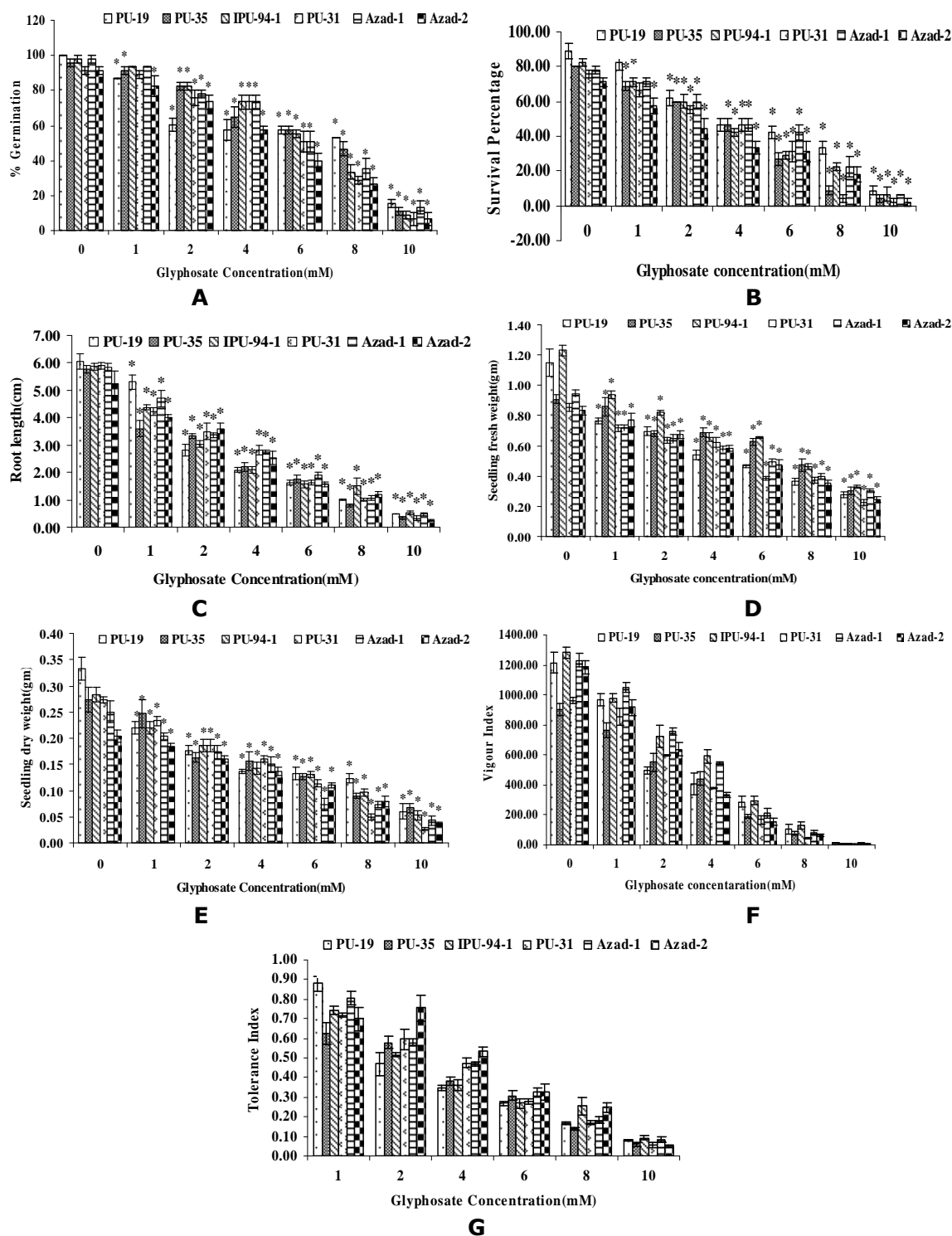


Figure.1: Effect of different Glyphosate concentrations on (A) Germination percentage (B) Survival percentage, (C) Root length (D) Fresh weight of seedlings (E) Dry weight of seedlings, (F) Seedling vigour index and (G) Tolerance index of six black gram genotypes. Vertical bars above mean denote standard error of three replicates. Means with asterisk differ significantly at 0.05 probability level by LSD test.

Discussion

Petridish bioassays are frequently used as the basis of germination test that allow simple and inexpensive analysis of biological activity of environmental pollutants, including herbicides and other hazardous agrochemicals (23, 24, 25). In this study effect of glyphosate on seed germination was assessed in six black gram genotypes and it was shown that even lowest concentration of glyphosate used was inhibitory for seed germination and root growth while at higher concentrations stunting of roots and reduction of root growth was observed. Already published reports have also mentioned that preharvest glyphosate applications lead to a general decrease in germination percentage. Preharvest application of glyphosate in pea reduced seed germination and seedling emergence (14). In a similar experiment, two *T. aestivum* varieties were assessed for effects of preharvest glyphosate treatment. Glyphosate was applied at 0.62 or 0.84 kg ae/ha at the milk, soft dough, or hard dough stage of wheat development. Glyphosate application at milk stage affected seed germination (15). Preharvest treatment of Derkado barley crops with glyphosate resulted in decreased levels of germination (16). Reduced seed germination due to glyphosate was also reported in *Cajanus cajan* (17). Inhibition of germination, radicle and plumule length by other herbicides like pendimethalin, alachlor and propachlor was also reported by many researchers (18, 19). Inhibition of germination is probably due to interference of herbicides with the metabolic activities related to it (19). In other finding it was reported that all pre and post-emergence herbicides had detrimental effects on rice seedling growth manifested in the form of reduced elongation of root and shoot and lower leaf and root score and dry biomass (20). Inhibition of germination was due to effect of herbicide on amylase activity and germination level (21). Our results also indicate that higher concentrations of herbicide-glyphosate inhibited the germination of black gram seeds at varying degree. This may be attributed to the adverse effect of the herbicide on degradation and mobilization of seed reserves. Inhibitory effect of herbicide stress on germination was also reported in *Chenopodium album* (22).

Glyphosate also showed adverse effect on seedling survival assessed 15 DAT. Other

reports also mention that glyphosate cause decrease in percent survival of soyabean and mungbean genotypes (27, 28). Sharp decrease in survival percentage caused by Diclofop-methyl (acetyl-co enzyme A carboxylase-inhibiting herbicide) was also reported in *Lolium rigidum* (26).

Many reports have clearly demonstrated that glyphosate also cause decrease in root length. The reason for reduced root length may be genotoxic effect of herbicide on root meristem. Herbicide induced inhibition of root length by herbicide in various crops have been reported by many workers (17, 29). Deleterious effect of glyphosate was observed on fresh weight of seedlings. Few studies also reported that the application of glyphosate led to a continuous decrease in the fresh weight of plants like maize (30), pea (14). Glyphosate has also been known to reduce leaf dry matter accumulation in *Phaseolus vulgaris* L. (Brecke and Duke, 1980). Deleterious effect of herbicide on seedling dry weight was observed at all concentration of herbicide in *Lolium multiflorum* (25). Abiotic stress causing decline in vigour index and tolerance index has been reported in many crops such as *Cicer arietinum* (32), spinach (13) and wheat (33, 34).

At highest dose germination percentage was greatest for PU-19 and Azad-1 and minimum for PU-31 and Azad-2 while survival percentage was greatest for PU-19 and minimum for PU-31 and Azad-2. Maximum fresh weight at 10mM was observed for IPU-94-1 and Azad-1 and minimum for PU-31 and Azad-2. Maximum dry weight at 10mM was observed for PU-19 and PU-35 and minimum for PU-31 and Azad-2. Maximum root length and tolerance index at 10mM was in IPU-94-1 and PU-19 and lowest in PU-31 and Azad-2. If all genotypes were compared on the basis of performance at higher doses 8mM and 10mM for all the parameters it was concluded that PU-19, IPU-94-1, PU-35 and Azad-1 are better performing varieties under herbicide stress than PU-31 and Azad-2.

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