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EVALUATION OF WEED CONTROL EFFICIENCY OF HERBICIDE RESISTANT TRANSGENIC COTTON

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ABSTRACT

Weeds incur significant losses to the crop plants by not only competing with crops for water, nutrients, sunlight, and space but also harbour insects and diseases. The herbicide resistant cotton offers farming community an opportunity to combat weeds by providing flexibility of herbicide application whenever needed. In the present study, the herbicide resistant cotton was evaluated to unravel the glyphosate dose response at different time intervals. Glyphosate was applied on advance transgenic cotton line VH-289 at the time intervals of 20, 25, 30 and 40 days after germination and was compared with manual weeding of its conventional non-transgenic counterpart as control. The weed control efficacy, types, mortality, and population of nineteen different weeds were calculated with reference to the time and dose of glyphosate spray [600 mL.acre $^{-1}$ (242 mL.ha^{-1}), 800 mL.acre $^{-1}$ (324 mL.ha^{-1}), 1000 mL.acre $^{-1}$ (305 mL.ha^{-1}), 1200 mL.acre $^{-1}$ (486 mL.ha^{-1}), 1400 mL.acre $^{-1}$ (566 mL.ha^{-1}), 1600 mL.acre $^{-1}$ (647 mL.ha^{-1}), 1800 mL.acre $^{-1}$ (728 mL.ha^{-1}) and 2000 mL.acre $^{-1}$ (809 mL.ha^{-1})] and its impact on yield. The yield and contributing characters like boll weight and cotton yield were higher in lines where glyphosate was applied at 20 and 30 days after germination compared to other treatment (manually hoed). Importantly, weeds control efficiency was recorded up to 98% at a dose concentration of 1800 mL.acre $^{-1}$ and 2000 mL.acre $^{-1}$ after 15-30 days after glyphosate application (DAS). The average number of bolls per plant in glyphosate resistant line was recorded higher compared to non-transgenic manually hoed control. This study highlights that spraying 1800 and 2000 mL.acre -1 glyphosate can help to eradicate weeds in field at less cost and good yield return. The results can be helpful for the cotton farmers regarding the proper concentration of glyphosate and its time of application.

Keywords: Herbicides, transgenic cotton, weed control efficacy, crop yield

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INTRODUCTION

Cotton is the main fiber crop that contributes significantly to the economy of many countries like China, United States, India, Brazil, and Pakistan (Zahid *et al*. 2016). Cotton faces many problems including insects, pests, and viruses but weeds are the most important ones reducing yield up to 40% (Awan *et al*. 2015). Weeds act as a competitor with crop plants and have proven to be the main cause of reduction of cotton yield both quantitatively and qualitatively (Ramesh *et al*. 2017). Besides that, weeds also serve as a reservoir for viral diseases (Bakhsh *et al*. 2020). To control weeds in cotton field, pre-emergence herbicides are sprayed extensively and still they have lesser weed control efficiency. In Pakistan, herbicides are extensively used, approximately at the rate of 16,495.00 tons per year causing an economic burden of $66 million USD (Economic Survey of Pakistan 2015-16). On the other hand, different practices like manual hoeing and mechanical weeds control have been deployed to eradicate weeds however it also causes damage to crop plants along with weeds.

Most of the available post-emergence herbicides are non-selective in nature with a considerable crop damage raising the need for development and testing of selective early post- emergence herbicides for weed control in cotton (Niu *et al*. 2017). Among these most successful herbicides is glyphosate. 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme regulates a crucial step in the biosynthesis of aromatic amino acids of the shikimate pathway, which are essential for plant growth (Filiz and Koc 2016). Although effective, but when this herbicide is sprayed on cotton plants for the removal of unnecessary weeds, it also causes adverse effects on cotton crop.

Many different types of strategies have been implemented to develop resistance against glyphosate; a few of them are primarily used such as over-expression of sensitive and insensitive forms of EPSP synthase (Tian *et al*. 2010) and glyphosate detoxification (Castle *et al*. 2004). Many glyphosate resistant transgenic cotton events have been developed, commercialized and are being cultivated on a very large acreage. The worldwide adoption of herbicide-tolerant cotton has gradually increased (about 10 % per year) and have resulted in increased farm productivity (Green 2012). However, very few studies show the dose response at different growth stages of transgenic cotton against glyphosate. In developing countries like Pakistan, the role of herbicide and glyphosate resistant cotton cultivars still requires further studies. In this study, an effort was made to express *CP4-EPSP synthase* in cotton cultivar VH-289 and to evaluate its efficacy at different time intervals, its dose effect and comparison with control.

MATERIALS AND METHODS

Genetic transformation of cotton with *CP4-EPSP* (GTG) synthase gene: The genetic transformation of cotton cultivar VH-289 was performed by shoot apex method as described earlier by Puspito *et al*. (2015). The transformation efficiency was calculated as reported by Bajwa *et al*. (2013).

Confirmation of gene integration and Expression: The transgenic cotton plants at T2 generations were screened for detection of *CP4-EPSP synthase* by PCR. Earlier, DNA was extracted from the transgenic and control plants using CTAB protocol. The sequence of primers used for the confirmation of putative transgenic plants was 5'-CGTGGGTGTGTATGACTTG-3' (*GTG* Forward Primer) and 5'-GTGTTGAGACCAGCGAGGAG -3' (*GTG* Reverse Primer) according to the protocol described by Latif *et al*. (2015). The amplified DNA fragment were resolved on 1 % agarose gel and images were documented.

For protein detection of transgenic cotton plants, enzyme linked immunosorbent assay (ELISA) was performed according to the protocol as described by Latif *et al*. (2015). The total protein was isolated from newly germinated leaves of herbicide resistant transgenic cotton plants along with control samples following protocol as described by Khan *et al*. (2011). The extracted protein form transgenic as well as control samples was transferred to 96 wells plate (provided along with kit) for binding with specific antibody generated against Glyphosate CP4-EPSPS. Rest of the procedure of assay was followed according to the instructions provided in the kit.

Glyphosate application assays in field and related data: Experimental area was divided into different sections to analyze the relation between growth of the cotton plants and amount of glyphosate spray. The different concentrations of glyphosate were applied at different growth stages (20, 25, 30 and 40 days after germination, DAG) of transgenic cotton plants along with manual hoeing of non-transgenic control cotton plants. The analysis of weed control efficiency was performed at different doses of glyphosate spray 600 mL acre^{-1} to 2000 mL. acre $^{-1}$.

Before this experiment, detailed examination of experimental area was performed to check the presence of weeds flora (Thomas *et al*. 2005; Subedi *et al*. 2017). Also, data of yield was collected from the plot where manual hoeing was performed to compare benefit ratio for the farmers.

Density of the weeds like grasses, sedges and broad-leaved weeds was recorded using 0.5m × 0.5m quadrates in each plot at 15 and 30 days after first spraying of glyphosate from four randomly fixed places as done by Andres *et al*. (2013) and Antralina *et al*. 2015). The weeds were collected by quadrate block method and categorized into sedges grasses and broadleaved. The weeds were dried in shade and then in hot-air oven at 80˚C for 72 hours. The dry weight of collected weeds was recorded separately at 15 and 30 days after first round of glyphosate spray and demonstrated in kg·ha^{-1} as described by Chauhan and Johnson (2010). Mechanical and hand weeding methods were used for the removal of weeds from cotton field.

For mechanical weed removal, wide varieties of tools were used including the conventional tractor drawn weeders. Most of the cotton growing farmers use one-to-two-time mechanical weed control including hand weeding in Pakistan. Usually, this practice is repeated more than once in a season depending on the intensity of weed infestation in the field.

Weed control efficiency (WCE) was calculated based on weed biomass of control and transgenic plots under control conditions. Weed control efficiency was calculated as described by Chauhan and Opeña (2013) and Ghosh *et al*. (2016).

Weed control efficiency (%): Weed density (control)-weed density (transgenic)*100/weed density control'

The important yield contributing traits like average boll number per plant and boll weight of transgenic and non-transgenic control plants were counted. Besides that, the cotton seed cotton yield (g) was also measured in transgenic plants in comparison with the control. All this data was collected and recorded as described by Bakhsh *et al*. (2009).

All data presented in the graphs are ($\bar{x}$ ± SD). Graph Pad Prism (Version 7) for windows was used for the data analysis. Data were subjected to analysis of variance to know if significant difference exists among the treatments. A significant difference was considered at $p < 0.05$.

RESULTS

A total of 54 putative transgenic cotton plants were obtained with transformation efficiency of 1.08% (based on selectable marker) and the efficiency of confirmed transgenic cotton plants was calculated as

0.1% based on molecular analyses. The transgenic cotton plants were acclimatized in soil pots under control conditions then shifted to field. A schematic diagram of transformation procedure is shown in Fig-1 as earlier described by our laboratory (Bajwa *et al*. 2013).

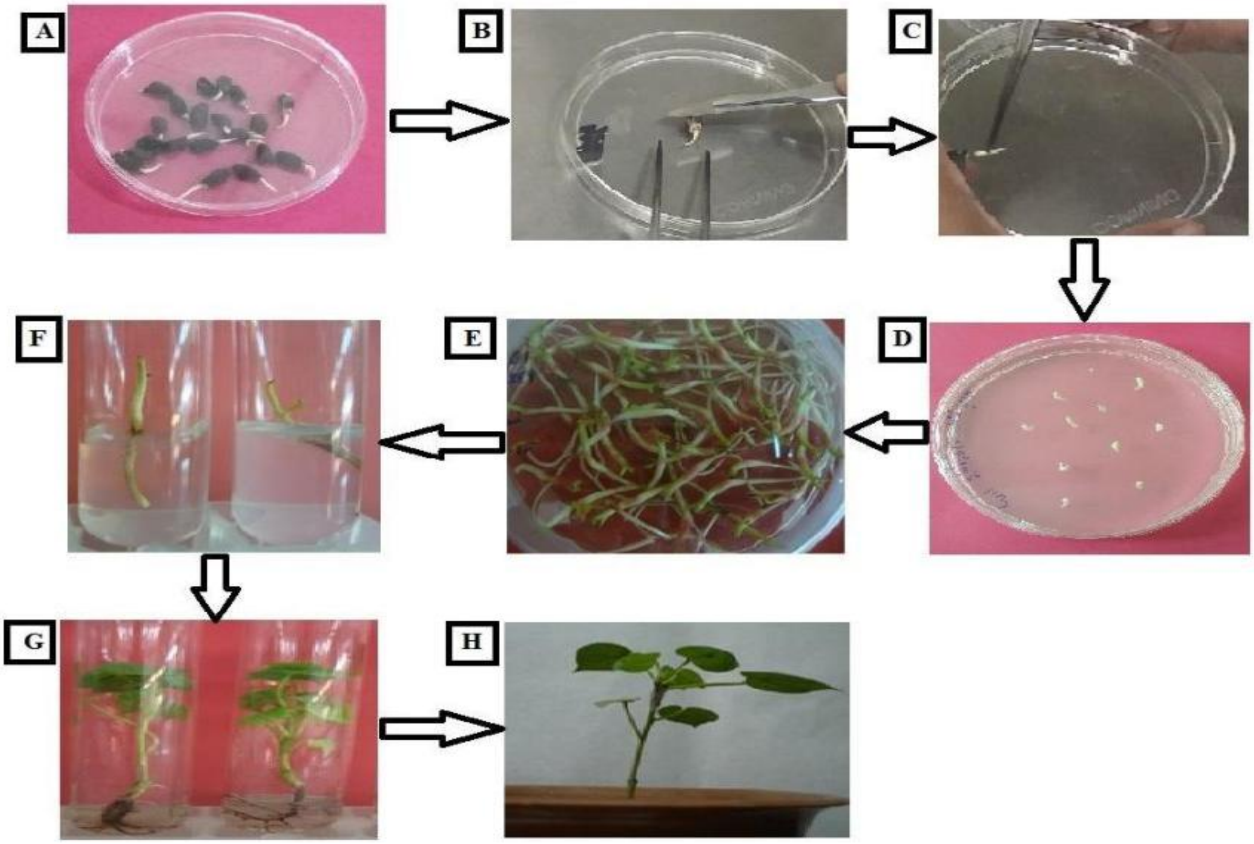

Fig 1: Stages of Transformation in cotton embryos, A: seed germination, B: isolation of embryos, C: injuring of cotton embryos, D: *Agrobacterium* infected embryos on MS medium, E: Agrobacterium treated embryos on MS medium after 3 days, F: implantation of cotton embryos in tube containing MS medium with kanamycin selection G: shoot induction under kanamycin selection, H: transgenic cotton plants in soil pots for acclimatization.

Gene specific primers of *CP4-EPSP synthase* gene were used for the amplification of internal 358 bp fragment in transgenic cotton plants in T2 (data now shown). Total 54 putative transgenic cotton plants were subjected to evaluation through PCR and 10 plants were found to have the transgene in T2 generation. No amplification was obtained for non-transgenic cotton plants. Likewise, PCR positive plants also showed expression of transgene when subjected to ELISA assays (data now shown).

To analyze the relation between growth of cotton plants and amount of glyphosate spray, experimental area was divided into four different sections having same transgenic lines (plants) of cotton cultivar VH-289. The different amount of glyphosate was applied to each section at 20,25,30 and 40 days following germination (Fig 2).

Weed Flora of Experimental Field: Nineteen different types of weeds were identified from experiment containment area, namely, broadleaf summer annual weeds, broad leaf winter annual weeds, grassy winter annual weeds, grassy summer annual weeds, broadleaf perennial weeds and grassy perennial weeds/Sedges. Weed flora in the experimental field predominantly comprised of twelve species of broad-leaved weeds, seven species of grasses and one type of sedge weed.

Among the broad-leaved weeds, grassy weeds were dominant like *Fimbristylis dichotoma, Leptochloa penicea, Paspalum distichum, Sorghum halepanse, Cyperus rotundus, Digitaria adscendense,* and *Trianthema portulacastrum (L.), Digera arvensis (*Forsk). *Cyperus rotundus* (L.) was the only sedge present in the experimental fields. Overall, Trianthema *portulacastrum, Digera arvensis, Euphorbia hirta, Portulaca oleracea, Euphorbia granulate, Euphorbia helioscpia, Convolvulus* *arvensis, Amaranthus viridis, Parthenium hyestrophorus, Trianthema portulacastrum, Tribulus terrestris, Xanthium spinosum* and *Phanera bariegata* were the dominant weed flora in the experimental field which results main losses because of its time-to-time growth after manual hoeing. *Trianthema portulacastrum* and *Cyperus rotundus* were found most common which compete with the resources of crop and can grow faster than cotton (Fig 3).

Fig 2: Field evaluation of transgenic cotton plants regarding same glyphosate spray at different time interval; A) glyphosate spray 20 days after germination (DAG) B) glyphosate spray 25 days after germination (DAG) C) glyphosate spray 30 days after germination (DAG) D) glyphosate spray 40 days after germination (DAG).

Weed control efficacy: Different doses of glyphosate spray such as 1600, 1800 and 2000 mL.acre $^{-1}$ applied at the time interval of 15 and 30 days significantly lowered the total weed density as compared to experimental field plots and mechanically hoed field crop. Lower doses of glyphosate at the rate of 600, 800 and 1000 mL.acre $^{-1}$ were ineffective in controlling total weeds and medium doses such as 1200 and 1400 mL.acre $^{-1}$ required many days for weed control (Fig 4A). First glyphosate spray controlled early season weeds and cotton field remained unchanged up to harvesting. After 30 days of first application of glyphosate at concentration of 2000, 1800 and 1600 mL.acre $^{-1}$, a lower dry weight of weeds was recorded due to better weed control at critical stage of crop growth in herbicide tolerant transgenic cotton. During the experimental period at 15 DAS, higher weed control efficiency of 98.78 % was recorded at 2000 mL.acre $^{-1}$ (Fig 4B). At 30 DAS, highest weed control efficiency (99.33 %) was observed at 2000 mL.acre $^{-1}$ followed by dose rate of 1600 (97.88 %) and 1800 mL.acre $^{-1}$ (98.86 %). While in manual hoed crop the weed control efficiency was recorded to be 57.71 % at 15 and 47.40 % at 30 days.

Data of morphological characteristics of transgenic cotton plants was collected in comparison to non-transgenic control with manual hoeing. The variation in all these characteristics depicted a correlation between agronomic characteristics and days of glyphosate spray. The more average number of bolls per plants were recorded in transgenic cotton plants compared to the non-transgenic control (Fig 5A). The average number of bolls (28-30) were recorded high in VH289-4 and VH289-6 lines following 40 days.

Besides that, boll weight of transgenic and non-transgenic cotton was also considered. Post glyphosate applications, boll weight of transgenic lines was found better as compared to control plants (Fig 5B). VH289-4 showed higher boll weight (4.2 g) compared to the other lines and control. The seed cotton yield of the transgenic plants was also recorded compared to conventional counterpart i.e., manually hoed non-transgenic control. Overall, all transgenic lines showed better seed cotton yield compared to the control, VH289-8 being the highest one post glyphosate applications at various intervals (Fig 6) followed by VH289-7 line.

Fig 3: Types of Weeds Present in Experiment Containment; A) ***Chenopodium album, B) Digera arvensis, C) Euphorbia hirta, D) Portulaca oleracea, E) Euphorbia granulate, F) Euphorbia helioscpia, G) Convolvulus arvensis, H) Fimbristylis dichotoma, I) Leptochloa penicea, J) Amaranthus viridis, K) Parthenium hyestrophorus, L) Trianthema portulacastrum, M) Tribulus terrestris, N) Paspalum distichum, O) Sorghum halepanse, P) Cyperus rotundus, Q) Digitaria adscendense, R) Xanthium spinosum, S) Phanera bariegata*****.**

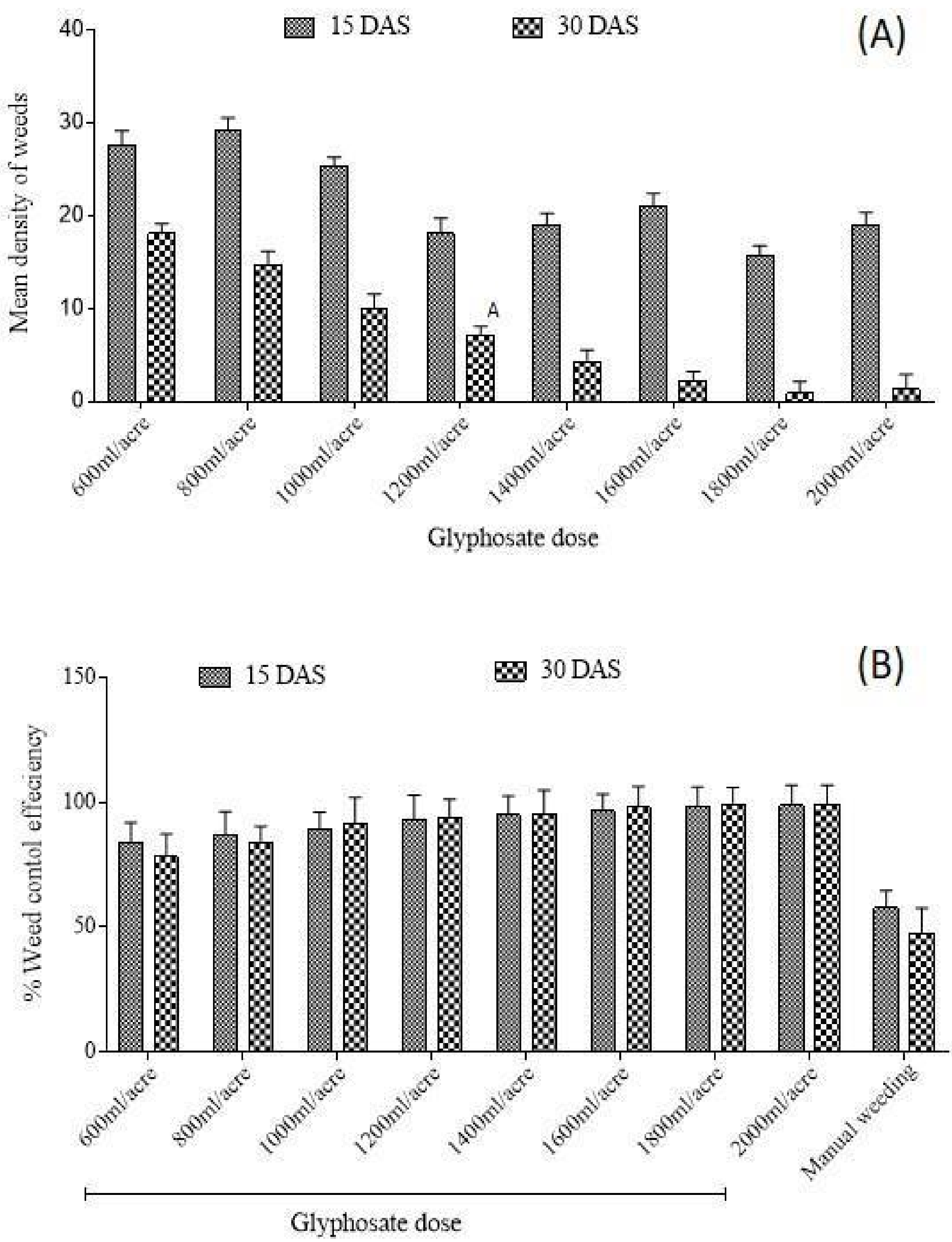

Fig 4: (A) weeds density after glyphosate spray (B) Efficacy of weed control (percentage) at the time interval of 15 and 30 days after spray (DAS)

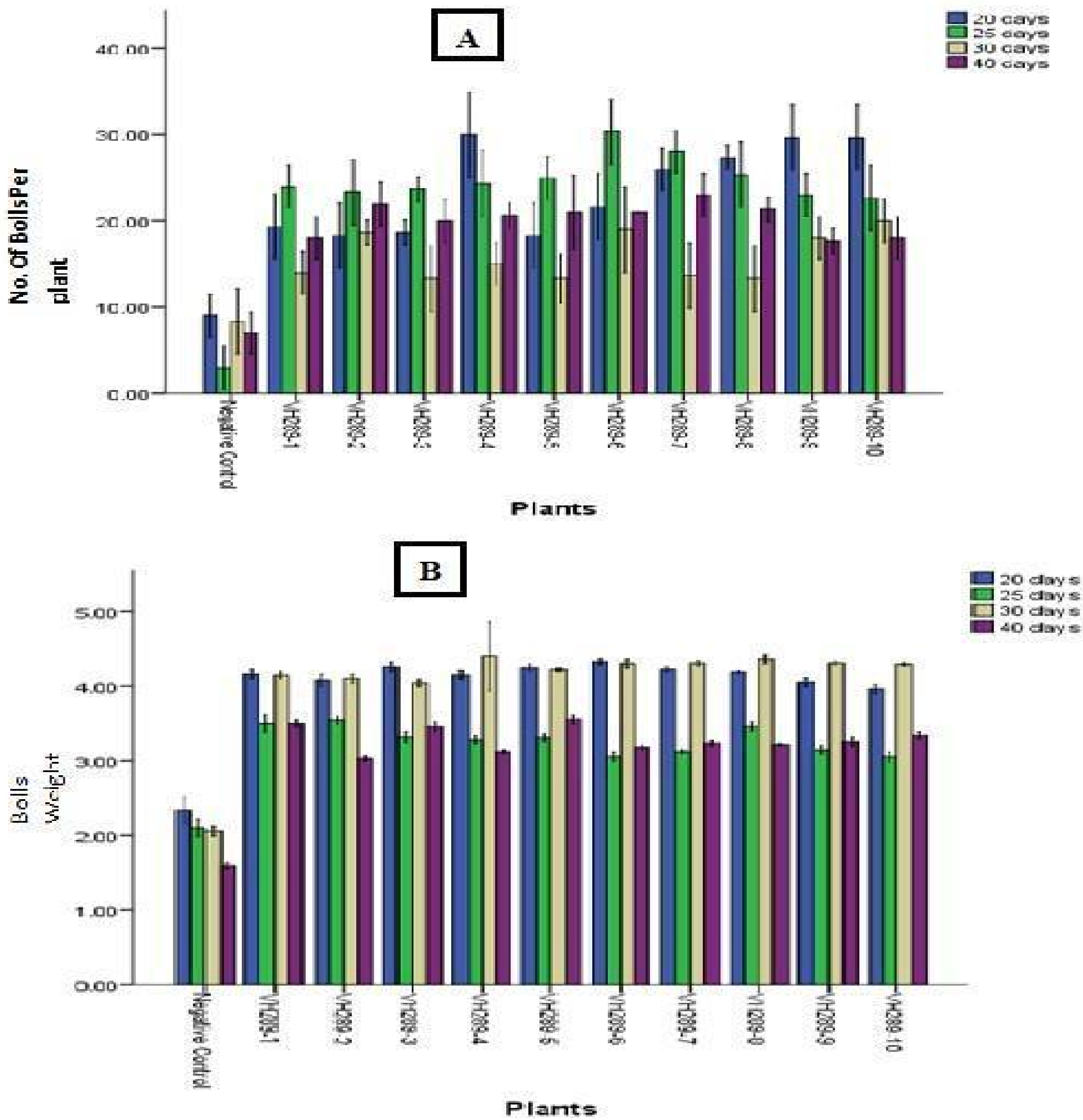

Fig 5: (A) Relationship between glyphosate spray and time of spray with no. of bolls per plant and (B) boll weight transgenic cotton plant in comparison to manually hoed control.

DISCUSSION

Cotton is an important cash and fiber crop with high economic value. The cotton yield losses incurred by weeds range up to 30%, moreover, weeds also act as a reservoir for insects and viruses. Application of pre-emergence herbicide and mechanical hoeing has been practiced but could not prove successful to overcome the losses (Arpat *et al*. 2004; Wilkins and Arpat 2005).

An efficient weed control approach has been reported by the introduction of *CP4-EPSP synthase* gene in local cotton cultivar VH-289 in current study. Molecular analysis confirmed that 10 out of the 54 putative transgenic cotton plants i.e., VH-289-1, VH-289-2, VH-289-3, VH-289-4, VH-289-5, VH-289-6, VH-289-7, VH-289-8, VH-289-9, and VH-289-10, in T2 generation as described by Puspito *et al*. (2015). These transgenic cotton plants were evaluated for glyphosate dose response in different time intervals and for comparison of yield losses saved with glyphosate application and mechanical hoeing.

Glyphosate was applied at four stages such as 20, 25, 30 and 40 DAG on transgenic cotton plants and same was done through mechanical hoeing in non-transgenic cotton plants. First four weeks of the emergence of plant is best time for weed control (Lassiter *et al*. 2007). In another part of this study, experimental area was divided into different sections for the measurement of correlations between different doses of glyphosate spray, its effects on morphological characteristics, application time in comparison with mechanical hoed cotton area.

Nineteen different types of weeds belonging to different taxonomical classes were identified such as annual and perennial grasses, sedges and broadleaf weeds as also identified by (Chinnusamy *et al*. 2013). Lower doses of glyphosate were ineffective in controlling some broad-leaved weeds like *Parthenium hysterophorus* and *Commelina benghalensis* and also *Cyperus rotundus*. Negligible or no control of weeds such as hemp *Sesbania*, *Ipomoea* species, hemp dogbane (*Apocynum cannabinum L*.), Bermuda grass (*Cynodon dactylon*), tropical spiderwort (*Commelina benghalensis* L.) and horse nettle (*Solanum carolinense* L.) was observed as it has already been as reported by Koger and Reddy (2005).

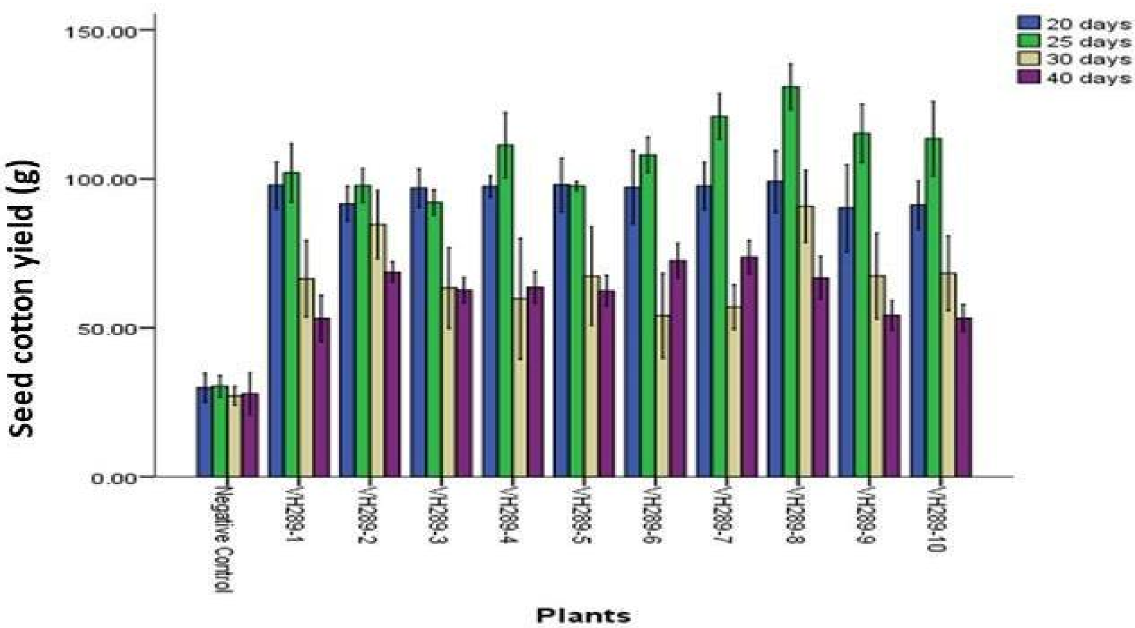

Fig 6: Seed cotton yield of transgenic cotton per plants with reference to time and dose of glyphosate spray

Better weed control (above 98 %) in the density of grasses, sedge and broad-leaved weeds was observed when glyphosate was applied at rate of 2000, 1800, 1600 mL.acre $^{-1}$ at the time of 15 days after herbicide spray because weeds were controlled at an early stage. Highest weed control efficiencies of 98.86 % (1800 mL.acre $^{-1}$) and 99.33 % (2000 mL.acre $^{-1}$) were recorded at 15 and 30 DAS, respectively. The better weed control efficiency of 97.88 % percent was observed at 1600 mL.acre $^{-1}$ concentration while 57.71 % and 47.40 % efficiency of weed control was obtained through mechanical hoeing. Glyphosate doses at the rate of 600 mL.acre $^{-1}$ and 800 mL.acre $^{-1}$ had lower weed control efficiency of 78.10 % and 83.54 % (Figure 6B). Grichar *et al*. (2004) and Main *et al*. (2007) also identified the response of cotton plants against glyphosate spray and reported that weed control efficiency is the main factor that directly affects the yield of cotton crop.

Insect and herbicide resistance transgenic cotton has contributed to better farm productivity with proper pest and weed management practices (Barpete *et al*. 2016). Weeds are natural competitors to crops in field and herbicide resistance has proven to be the most effective remedy against these with minimal labor (Green 2012). Yield is the goal from crops in reduced price and labor. Many studies have already been carried out about use and safety of glyphosate. In this study we report the glyphosate dose efficiency and time of application with reference to crop stage in quantitative terms. Herbicide tolerant transgenic cotton gave higher yield with glyphosate at 2000 mL. acre $^{-1}$ as compared to hand weeding and un-weeded control. Similarly, seed cotton yield was found maximum after the glyphosate spray at different growth stages which indicates the efficient way of weed control leads to better yield as well (Scroggs *et al*. 2007). Post glyphosate applications, the transgenic lines showed better weed control, number of bolls, boll weight and yield compared to the controls (Fig 5-6) indicating the robustness of the technology, addressing main concerns of weed control in small as well as large scale seed production blocks.

From the data obtained, it can be interpreted that with the use of herbicide resistant cotton, weed management in crop can be performed better compared to mechanical manual hoeing. Time and dose of glyphosate spray is the key to maximize the potential of crop yield. Our data showed that spraying 1800 and 2000 mL.acre $^{-1}$ glyphosate can help to eradicate weeds in field at less cost and good yield return following 25-30 days post glyphosate application.

Conclusion: The study was conducted to investigate the glyphosate dose response at different time intervals on advance transgenic cotton line VH-28 compared with manual weeding of its conventional non-transgenic counterpart as control. The results of this study not only provide important information to the scientific community but equally important to farmers who are the stakeholder of this technology.

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Author Contribution: RAQ, KSB and MAA contributed equally to the manuscript in all experiments. AI and AL performed molecular analysis. AB drafted the article and edited it. RAQ, TH, IAN and AAS conceptualized the study, designed experiments, and supervised the overall study and presented manuscript in its current form.

Competing interests: The authors declare that they have no competing interests.

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REFERENCES

Andres, A., G. Concenço., G. Theisen., F. Vidotto and A. Ferrero (2013). Selectivity and weed control efficacy of pre-and post-emergence applications of clomazone in Southern Brazil. Crop Prot. 53:103-108.

Antralina, M., I.N. Istina and T. Simarmata (2015). Effect of difference weed control methods to yield of lowland rice in the SOBARI. Procedia Food Sci. 3:323-329.

Arpat, A., M. Waugh., J.P. Sullivan., M. Gonzales., D. Frisch., D. Main., T. Wood., A. Leslie., R. Wing and T. Wilkins (2004). Functional genomics of cell elongation in developing cotton fibers. Plant Mol. Biol. 54**:**911-929

Awan, M.F., M.A. Abass., A. Muzaffar., A. Ali., B. Tabassum., A.Q. Rao., I.A. Nasir, I and T. Husnain (2015). Transformation of insect and herbicide resistance genes in cotton (Gossypium hirsutum L.). J. Agric. Sci. Technol. 17:287-298.

Azhar, M.T., R.M. Atif., M. Israr., I.A. Khan., S. Khalid and I.A. Rana (2021). A discussion on cotton transformation during the last decade (2010–2021); an update on present trends and future prospects. J Cotton Res. 4(1):1-14.

Bajwa, K.S., A.A. Shahid, A.Q. Rao., M.S. Kiani., M.A. Ashraf., A.A. Dahab., A. Bakhsh., A Latif., M.A.U. Khan., A.N. Puspito and A. Aftab (2013). Expression of Calotropis procera expansin gene CpEXPA3 enhances cotton fibre strength. Aust. J. Crop Sci. 7:206.

Bakhsh, A., A.Q. Rao., A.A. Shahid., T. Husnain and S. Riazuddin (2009). Insect resistance and risk assessment studies in advance lines of Bt cotton harboring Cry1Ac and Cry2A genes. Am Eurasian J Agric Environ Sci. 6(1):1-1.

Bakhsh, A., T. Hussain., I. Rahamkulov., U. Demirel and M.E. Çalışkan (2020). Transgenic potato lines expressing CP4-EPSP synthase exhibit resistance against glyphosate. Plant Cell, Tiss Organ Cult. 140:23-34.

Barpete. S., A. Bakhsh., E. Anayol., S.F. Özcan, M.C. Oğuz., O.M. Karakoç and S. Özcan (2016). Inducing osmotic stress leads to better genetic transformation efficiency in Cotton (*Gossypium hirsutum* L.). Turk J Biol. 40: 826-836.

Castle, L.A., D.L. Siehl., R. Gorton., P.A. Patten., Y.H. Chen., S. Bertain. H.J. Cho., N. Duck., J. Wong., D. Liu and M.W. Lassner, M.W (2004). Discovery and directed evolution of a glyphosate tolerance gene. Sci. 304:1151-1154.

Chauhan, B and J. Opeña (2013). Weed management and grain yield of rice sown at low seeding rates in mechanized dry-seeded systems. Field Crops Res. 141:9-15.

Chauhan, B.S and D.E. Johnson (2010). The role of seed ecology in improving weed management strategies in the tropics. Adv. Agron. 105**:**221-262.

Chinnusamy, N., C. Chinnagounder and P.N. Krishnan (2013). Evaluation of weed control efficacy and seed cotton yield in glyphosate tolerant transgenic cotton. Am J Plant Sci. 24:1159.

Economic survey of Pakistan 2015-16. Agriculture Chapter #2. Ministry of Finance, Govt. of Pakistan.

Filiz. E and I. Koc (2016). Genome-wide identification and comparative analysis of EPSPS (aroA) genes in different plant species. J Plant Biochem Biotechnol. 25**:**21-29.

Ghosh D, Singh UP, Ray K, *et al*. Weed management through herbicide application in direct- seeded rice and yield modeling by artificial neural network. Span. J. Agric. Res. 2016;14:

Ghosh, D., U.P. Singh.,K. Ray and A. Das (2016). Weed management through herbicide application in direct-seeded rice and yield modeling by artificial neural network. Span J Agric Res. 14: pp e1003-e1003.

Green, J.M. (2012). The benefits of herbicide-resistant crops. Pest Manag. Sci. 68:1323-1331.

Grichar, W.J., B.A. Besler., K.D. Brewer and B.W. Minton (2004). Using soil-applied herbicides in combination with glyphosate in a glyphosate-resistant cotton herbicide program. Crop Prot.23(10):1007-1010.

Khan, G.A., A. Bakhsh., S. Riazuddin and T. Husnain (2011). Introduction of cry1Ab gene into cotton (Gossypium hirsutum) enhances resistance against Lepidopteran pest (Helicoverpa armigera). Span. J. Agric. Res. 1:296-302.

Koger, C.H and K.N. Reddy (2005). Glyphosate efficacy, absorption, and translocation in pitted morningglory (Ipomoea lacunosa). Weed Sci. 53**:**277-283.

Lassiter, B.R., I.C. Burke., W.E.Thomas., W.A. Pline-Srnić., D.L. Jordan., J.W. Wilcut and G.G. Wilkerson (2007). Yield and physiological response of peanut to glyphosate drift. Weed Technol. 21(4):954-960.

Latif A, A. Q. Rao, M. A. U. Khan, N. Shahid, K. S. Bajwa, M. A. Ashraf, M. A. Abbas, M. Azam, A. A. Shahid, I. A. Nasir and T. Husnain (2015). Herbicide-resistant cotton (*Gossypium hirsutum*) plants: an alternative way of manual weed removal. BMC Res. Notes 8:453.

Latif, A., A.Q. Rao., M.A.U. Khan, N. Shahid., K.S. Bajwa., M.A. Ashraf., M.A. Abbas., M. Azam., A.A. Shahid., I. A. Nasir and T. Husnain (2015). Herbicide-resistant cotton (Gossypium hirsutum) plants: an alternative way of manual weed removal. BMC Res. Notes 8:453

Main, C.L., M.A. Jones and E.C. Murdock (2007). Weed response and tolerance of enhanced glyphosate-resistant cotton to glyphosate. J. Cotton Sci. 11**:**104-109.

Niu, L, W. Ma, C. Lei, J. L. Jurat-Fuentes and L. Chen (2017). Herbicide and insect resistant Bt cotton pollen assessment finds no detrimental effects on adult honey bees. Environ. Pollut. 230**:**479-485.

Puspito, A.N., A.Q. Rao., M.N. Hafeez., M.S. Iqbal., K.S. Bajwa ., Q. Ali., B. Rashid., M.A. Abbas., A. Latif., A. A. Shahid and I. A. Nasir (2015) Transformation and evaluation of Cry1Ac+ Cry2A and GTGene in Gossypium hirsutum L. Front. Plant Sci. 6:943.

Ramesh, K., A. Matloob., F. Aslam., S.K. Florentine and B.S. Chauhan (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. Front. Plant Sci. 2017; 8:95.

Scroggs, D.M., D.K. Miller., J.L. Griffin., J.W. Wilcut., D.C. Blouin., A.M. Stewart and P.R.Vidrine (2007). Effectiveness of pre-emergence herbicide and postemergence glyphosate programs in second-generation glyphosate-resistant cotton. Weed Technology, 21(4):877-881.

Subedi, M., C.L. Willenborg., A. Vandenberg (2017). Influence of harvest aid herbicides on seed germination, seedling vigor and milling quality traits of red lentil (*Lens culinaris* L.). Front. Plant Sci. 8;311.

Thomas, W.E., I.C. Burke., B.L. Robinson., W.A. Pline-Srnić., K.L. Edmisten., R. Wells and J.W. Wilcut, (2005). Yield and physiological response of nontransgenic cotton to simulated glyphosate drift. Weed Technol. 19(1):35-42.

Tian, Y.S., A.S. Xiong., J. Xu., W. Zhao., F. Gao., X.Y. Fu., H. Xu., J.L. Zheng., R.H. Peng and Q.H.Yao (2010). Isolation from Ochrobactrum anthropi of a novel class II 5-enopyruvylshikimate-3-phosphate synthase with high tolerance to glyphosate. Appl Environ Microbiol. 76(17):6001-6005.

Wilkins, T.A and A.B. Arpat (2005). The cotton fiber transcriptome. Physiol Plant. 124**:**295-300.

Zahid, K. R., F. Ali., F. Shah., M. Younas., T. Shah., D. Shahwar., W. Hassan., Z. Ahmad., C. Qi., Y. Lu and A. Iqbal (2016). Response and tolerance mechanism of cotton Gossypium hirsutum L. to elevated temperature stress: a review. Front. Plant Sci.; 7:937.