Chemical Science Review and Letters ISSN 2278-6783

Review Article

Weed Management in Herbicide Tolerant Transgenic Maize -A Review

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Abstract

Weeds and crops compete for the same resources of nutrients, water, space and light for growth and development. Weeds are generally hardy species having fast growth, deep root system and capable of competing very efficiently with cultivated crops for the available resources and adversely affect the crop growth and yield. Initial slow growth particularly at early crop growth stages and wider plant spacing of maize crop encourages fast and vigorous growth of weeds. It is paramount important that, competition from weeds must be minimized to achieve optimum yield. Among the different weed control methods, chemical method bears many advantages in suppressing weed growth and to get healthy and vigorous crop stand. Over the last few decades, many classes of selective and non-selective herbicide molecules with a variety of mode of action were discovered. Some excellent non-selective herbicides could be used only when the crop was not present in the field. At present days modern biotechnological tool brought excellent invention to agriculture by introduction of transgenic crops that made resistant to non-selective herbicides. The broad spectrum of activity, good crop growth and environmental safety of glyphosate are likely to result in attempting total post emergence weed control using glyphosate resistant maize. A brief review on weed flora of maize, methods of weed control, transgenic herbicide resistant crops and the effect of glyphosate resistant maize on weed management, growth, yield and quality characters are reviewed.

Keywords: Transgenic maize, Herbicide tolerance, Weed management, Glyphosate, Herbicide

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Introduction

Maize is one of the most important cereal crops cultivated worldwide and it is not only an important human nutrient source, but also a basic element of animal feed and raw material for manufacture of many industrial products, besides being recently used as biofuel. Maize is a versatile crop having higher yield potential among cereals and cultivated over a wide range of agro climatic zones and hence it is popularly called as "*Queen of Cereals*". Average maize productivity in United States and Spain is over 9.0 metric tonnes, Brazil is about 3.75 metric tonnes and India is only 2.5 metric tonnes. India needs to increase growth rate of maize by 9.51 per cent to meet the increasing demand. One of the possible ways to bridge the gap between demand and supply is to increase the productivity per unit area by adopting appropriate management technologies. The major yield reducing factors for maize cultivation in India are weeds and insects. Weeds cause considerable yield loss due to competition for resources with maize crop. Season long competition reduced the grain yield of maize in as much as 70 per cent [1]. Therefore, weed management is an important agronomic practice to ensure optimum grain yield. Weed management in maize is carried out by manual, mechanical and chemical methods, among which chemical method is the most economical and effective tool to suppress weeds in order to get healthy crop stand and good yield.

Genetic engineering is one form of biotechnological tool that is used to enhance the agronomic characteristics of plants by inserting a gene or sequence of genes that express desirable traits. The most successful example has been glyphosate-resistant technology. The ability to manipulate the plant genome directly gave scientists new ways to create maize crop tolerant to glyphosate. The introduction of glyphosate-resistant crops has created new opportunities for the use of effective, non-selective herbicide like glyphosate as selective weed control in crop production. Prior to the introduction of glyphosate-resistant crops, glyphosate is being applied to control existing vegetation prior to sowing of crops. Now, it can be used as a post emergence herbicide in crops like soybean, cotton, canola and maize [2]. Hence, a brief review is presented on weed flora of maize, methods of weed control, transgenic herbicide resistant crops and the effect of glyphosate resistant maize on weed management, growth, yield and quality characters

Weed spectrum in maize

Effective and economical weed management depends on a detailed knowledge on the types of weed flora under specific environmental conditions of the location and soil type. Weed flora of a location varies depending upon soil type and environment. Therefore, proper identification of weed species prevailing in the field is essential for implementing any weed control programme successfully. Weed flora of the experimental field at initial stage of crop growth was dominated by grassy weeds (47.7 per cent) consisting of *Cynodon dactylon* (21 per cent), *Dactyloctenium aegyptium* (15.8 per cent) and of major broad leaved weeds *Digera arvensis* (18.5 per cent), *Trianthem portulacastrum* (10.8 per cent) while *Cyperus rotundus* was the major sedge weed (10.2 per cent) [3]. Further a shift in weed flora over growth stage of the crop was also observed. The initial density of grasses (47.7 per cent at 30 DAS) was reduced to 15.8 per cent at maturity stage of the crop, while broad leaved weeds population increased from 39.5 to 69.6 per cent. According to [4] the major weed flora of maize field in Tamil Nadu were *Cynodon dactylon, Dactyloctenium aegyptium* among grasses, *Parthenium hysterophorus, Trianthema portulacastrum* and *Digera arvensis* among the broad leaved weeds and *Cyperus rotundus* as the only sedge weed. The major weed flora observed in the experimental plots of Bangalore was *Cynodon dactylon, Digitaria marginata, Dactyloctenium aegyptium, Eragrostis pilosa, Eragrostis riparia* and *Panicum* spp. among grasses, sedge *Cyperus rotundus* and *Aegratum conyzoides, Amaranthus viridis, Acanthospermum hispidum, Mimosa pudica, Phyllanthus niruri, Portulaca oleracea* and *Cleome monophylla* among broad leaved weeds [5].

Experimental fields in Turkey, average weed ground cover at the 3 leaf stage of maize were 10 per cent for *C. rotundus*, 5 per cent for *A. retroflexus*, 3 per cent for*C. album* and 2 per cent for *P. oleracea* (total weed ground cover was 20 per cent) [6] Similarly, [7] documented that the experimental fields of Annamalainagar was infested mainly with *Cyperus rotundus, Trianthema portulacastrum* and *Cleome viscosa.*Experimental fields of Faisalabad consisted with three major weeds, *viz., Trianthema portulacastrum, Cyperus rotundus* and *Coronopus didymus* [8]. The most common weeds in the experimental field at southern east of Iran were *Chenopodium album, Salsola kali, Heliotropium europaeum* and *Alhagi pseudalhagi.* These four species represented nearly 80 per cent of the total weed population. *Chenopodium album* and *Salsola kali* were the most predominant species and accounted for more than 50 per cent of the weed populations [9].

Critical period for weed control

The critical period for weed control is a key component of an effective weed control program. It is a period in the crop growth cycle during which weeds must be controlled to prevent yield losses. The critical period for weed control is useful for making decisions on the need for and timing of weed control. Timing of weed control measures is important to maintain optimum crop yield. Determining the critical period for weed control could help reduce yield losses due to weed interference. [10] Reported more severe weed competition upto 30 to 40 days after crop emergence. Under Mid-hill condition of North-Western Himalayas the first 40 days after emergence was found to be critical for weed competition [11]. [12] Also reported that the critical period of competition between crop and weed was between 30 and 45th day after sowing. Studies conducted to evaluate the critical period of weed removal and the effect of period of weed removal and the effect of weed competition on maize showed that 20 to 60 days after sowing was observed to be the critical period [13]. In Canada, [14] showed that the critical period for weed control in maize began at the three leaf stage and ended at the fourteenth leaf stage. [15] Notified that late weeding results in crop losses, especially if it is carried out after the critical period of weed competition. Maize can withstand weed competition upto3-4 weeks early in the growing season and weeds that emerge at 6-9 weeks after planting (WAP) do not cause significant maize yield losses. [16] Suggested that weed-free period between the three and seven to ten leaf stages of the maize was enough to prevent the yield losses under the growing conditions of Aydin province, Turkey. Another study conducted in Turkey, showed that the critical period for weed control was 5 week, which corresponded to the one to five leaf stage of maize [17]. [18] Observed that, under experimental conditions maize tolerates weed interference until 19 DAE suggesting that control measures should start at that stage. The crop should be kept weed free until 55 DAE in order to prevent yield loss in excess of 5 per cent.

Methods of weed management in maize
Manual and cultural methods

Weed control is one of the most important objectives of cultural operations in crop production. These methods include controlling weeds either by manual labour, bullock drawn implements or by power driven machinery. Field experiments conducted at Hamiput of Himachal Pradesh showed that hoeing and manual weeding twice increased maize grain yields by 0.36 t ha^{-1} [19]. Similarly, at Ranchi, adoption of hand weeding at 20 and 40 DAS resulted in minimum weed infestation in maize during rainy season [20]. Hand weeding at 20 and 40 DAS in rainfed maize gave the highest weed control efficiency, which was comparable with metolachlor (1.25 kg ha^{-1}) in Bajaura region of Himachal

Pradesh [21]. Application of atrazine at 7 and 14 DAS and hand weeding at 25 and 50 DAS produced statistically similar stover production compared to that of season long weed free treatment [22]. Results of the field experiment conducted by [23] showed that hand weeding significantly reduced the density and dry matter of weeds compared to chemical treatment in maize cultivated on clay loam soils of Chittorgarh region.

Lower total weed population due to hand weeding treatments in maize-chickpea cropping system [24]. Earthing up at 30 DAS resulted in the virtual elimination of weeds throughout the crop growth period [25]. [26] Recorded higher WCE of 82.2 per cent with two hand weedings done at 15 and 30 DAS. [27] reported that manual weeding twice registered reduction of *C. rotundus* (22.2 per cent), *D. arvensis* (17.6 per cent) *P. niruri* (22.0 per cent) and *C. benghalensis* (20.3 per cent) population resulting in heavy decline in weed dry weight and higher weed control efficiency (87.8 per cent) in rainy season maize in Central Uttar Pradesh. Two weedings recorded 87.55 per cent WCE and one weeding *fb* earthing treatment recorded maximum of 93.88 per cent WCE at 45 DAS indicating suppression of first flush of weeds successfully in maize [28].Wiltshire *et al.* (2003) reported that plant population density was not affected by mechanical weeders associated with chemical weeding, at the same time, higher weed control efficiency was achieved compared to hand weeding. [29] Noticed that hand weeding twice at 3 and 6 weeks after sowing showed significant effect in reducing weeds and also increased the yield when compared with weedy check.

Chemical method

Number of researchers reported positive response in maize growth and yield due to chemical weed control methods. [30] Reported that herbicides significantly increased maize yield and decreased the weed density. [31] reported that, herbicide application proved effective in controlling weeds and increasing the grain yield of maize in Peshawar, Pakistan.

Pre-emergence herbicides

Atrazine is a selective systemic herbicide having both knockdown effects and residual activity. It is one of the most popular herbicides in World, despite criticisms against its continued use in crop fields. According to[32], application of atrazine at 2.0 kg a.i ha^{-1} proved quite effective in controlling weeds (WCE 67 per cent) and enhancing grain yield of maize by 143 per cent (3636 kg ha) over control (1351 kg ha^{-1}). [33] Reported that pre-emergence application of atrazine at 0.5 kg ha^{-1} completely controlled the broad leaved weeds, but all grassy weeds were not controlled. According to [34] application of atrazine at 0.25 or 0.5 kg ha^{-1} on 7 or 14 DAS proved either equally or more effective than its pre-emergence application against *Trianthema portulacastrum, Echinochloa colonum* and *Digera arvensis*. [35] Reported that pre-emergence application of simazine (1.0 kg ha^{-1}) or atrazine (1.0 kg ha^{-1}) produced maize grain yield equal to that of weed free condition. Atrazine was more effective against *Ageratum conyzoides* and less effective against *Echinochloa colonum* and *Brachiaria romosa* than pendimethalin or alachlor [36]. [37] Concluded that atrazine behaviour in the soil is influenced by soil type, pH, temperature, and organic matter content and moisture availability.

Pre-emergence application of atrazine at 0.5 kg ha^{-1} followed by inter cultivation at 35 DAS increased the maize grain yield by 93 per cent over weedy check. The yield under this treatment was comparable with inter cultivation at 20 and 35 DAS in clay loam soils of Udaipur [38]. [39] Concluded that higher maize equivalent was recorded in maize + soybean intercropping applied with pre-emergence alachlor at 1.5 kg ha^{-1} followed by hand weeding (8803 kg ha^{-1}). This was closely followed by maize + soybean with alachlor at 1.5 kg ha^{-1} alone (8103 kg ha^{-1}). [40] reported minimum dry weed biomass (29.55 g m^{-2}) in ridge planting of maize with application of metolachlor as pre-emergence as against maximum dry weed biomass (88.75 g m^{-2}) under broadcast sowing and weedy check and also maximum plant height (213.42 cm). Maximum leaf area (349.00 cm^{2}) and maximum biological yield of 10.67 t ha^{-1} recorded in metolachlor treated plots against weedy check treatment. Ease of availability, low price and efficiency are the factors contributing to its popularity [41]. The extensive and continued use of atrazine and metolachlor mainly in maize production has led to these herbicides being detected in surface and groundwater resources throughout the southeastern Coastal Plains. Because of the potential negative impact of atrazine and metolachlor on water quality, alternative methods of weed control are needed that will result in similar or higher crop productivity and provide the same or greater level of weed control.

Transgenic crops

The advent of modern technology through the application of biotechnology on plants is a positive and innovative step in accelerating agricultural development worldwide especially in the developing countries like India. Biotechnology brought big business and huge investments into agriculture and revolutionized the way biologists do research [42]. Popularly known genetically modified organisms (GMOs), are organisms in which the genetic material (DNA) has been altered from natural processes. The technology is often called “gene technology” or “genetic engineering”. While some of the developed countries, especially the United States, Australia, Canada and the European Union have long been using genetic engineering in the agricultural production. Since the first reports of transgenic plants appeared in 1984, there has been very rapid progress directed at using this new technology for the practical ends of crop improvement. Protection of crops from insect pests was quickly seized upon as a major goal of plant genetic engineering. The potential size of this market attracted major attention from a number of commercial organizations and the potential economic importance of this sector of biotechnology is finally becoming more widely recognized [43].

The growth rate of transgenic crops between 1996 and 2008 was an 74 fold increase and making it the fastest adopted crop technology in recent history. In 2008, the global hectarage of biotech crops continued to grow strongly reaching 125 million hectares, up from 114.3 million hectares in 2007. The number of countries to cultivate biotech crops has increased steadily from 6 in 1996, the first year of commercialization to 18 in 2003 and 25 in 2008. Biotech soybean continued to be the principal biotech crop in 2008, occupying 65.8 million hectares or 53 per cent of global biotech area, followed by biotech maize (37.3 million hectares or 30 per cent), biotech cotton (15.5 million hectares or 12 per cent) and biotech canola (5.9 million hectares or 5 per cent) of the global biotech crop area. In the same year number of farmers benefiting from biotech crops globally across 25 countries reached 13.3 million, an increase of 1.3 million over 2007 remarkably over 90 per cent or 12.3 million (up from 11 million in 2007) were small and resource-poor farmers from developing countries [44]. In India, 5 million small farmers benefited from planting 7.6 million hectares of Bt cotton during 2008. An average, conservative estimates for small farmers indicate that yield increased by 31 per cent, insecticide application decreased by 39 per cent and profitability increased by 88 per cent equivalent to Rs.12, 500 per hectare.

Herbicide tolerant crops

Herbicide-tolerant plants are produced by the stable insertion of a gene that expresses a modified plant synthase protein in the receptor plant that is tolerant to particular herbicides [45]. In the early 1980s, the tools for producing transgenic crops were becoming available. Introduction of transgenic crops made resistant to broad-spectrum, non-selective herbicides was rightfully perceived as a better strategy in terms of weed management and market share. Several companies saw the advantage of using this technology to produce crops resistant to very broad spectrum herbicides. The two herbicides that fitted this approach best were glyphosate and glufosinate. Both compounds are amino acid analogues that have molecular targets in amino acid biosynthesis pathways. Herbicide-resistant crops were the first major wave of transgenic crops [46].

From the genesis of commercialization in 1996 to 2008, herbicide tolerance has consistently been the dominant trait. In 2008, herbicide tolerance deployed in soybean, maize, canola, cotton and alfalfa occupied 63 per cent or 79 million hectares of the global biotech area of 125 million hectares. For the second year running in 2008, the stacked double and triple traits occupied a larger area (26.9 million hectares, or 22 per cent of global biotech crop area) than insect resistant varieties (19.1 million hectares) at 15 per cent (James, 2009). Wide-spread adoption of GR crops and glyphosate has had significant economic effects in agriculture, from replacement of previous herbicide markets [47] to cost savings for farmers in weed management (Brookes and Barfoot, 2008). Furthermore, GR crop technology has generally reduced the adverse environmental and health impacts of weed management [48].

Glyphosate tolerant crops

Development of glyphosate resistant crop technology

Glyphosate is a foliar applied, broad spectrum, post emergence herbicide capable of controlling annual and perennial grasses and dicotyledonous weeds [49]. Glyphosate was classified as a herbicide after it was discovered by J.E. Franz in 1971 at Monsanto and was commercialized under the trade name Roundup. Today, glyphosate is sold as an isopropylamine salt, trimethylsufonium (trimesium) salt, sesquisodiumsalt, potassium salt and ammonium salt under several hundred trade names by Monsanto and other chemical companies. The mode of action of glyphosate is inhibition of aromatic amino acid biosynthesis specifically inhibition of 3-phospho-5-enolpyruvylshikimte synthase (EPSPS) which reduces the plant’s ability to form aromatic amino acids such as tryptophan, tyrosine, phenylalanine and other important secondary compounds. Glyphosate is a foliar applied herbicide which once absorbed is readily translocated in the xylem and phloem throughout the plants with primary sinks being actively growing vegetative tissue and reproductive tissue; however, it has no soil residual activity [50].

In 1983, scientists at Monsanto and Washington University isolated the common soil bacteria, *Agrobacterium tumefaciens* strain CP4, which is highly tolerant to glyphosate because its EPSPS is less sensitive to inhibition by glyphosate than EPSPS found in plants [51]. When plants expressing the CP4 EPSPS proteins are treated with glyphosate, the plants continue to grow. The continued action of the tolerant CP4 EPSPS enzyme provides the plant's need for aromatic acids. Aromatic amino acid biosynthesis is not present in animals. This explains the selective activity in plants and contributes to the low mammalian toxicity of glyphosate. By 1986, they had successfully inserted the CP4 EPSPS gene into the plant genome and obtained GR plants. Within 10 year, GR soybean was commercialized. This gene transformation resulted in soybean plants resistant to high levels of glyphosate. This event was patented as the Roundup Ready gene technology, expressed in soybeans and released into the commercial marketplace in 1996 [52]. The initial GR crops were the most quickly adopted technology in the history of agriculture. This rate of adoption continues at more than 10 per cent per year in both developing and developed countries. The introduction of GR crops transformed the way many growers manage weeds. Growers chose GR crops because glyphosate made weed control easier and more effective, increased profit, required less tillage, and did not restrict crop rotations. Glyphosate-resistant crops approved for sale in the USA include canola, corn (*Zea mays*), cotton (*Gossypium hirsutum* and *G. barbadense*), soybean (*Glycine max*) and sugarbeet (*Beta vulgaris*).

Glyphosate tolerant maize

Modern technologies introduce the new approaches to weed management systems in maize that include the use of post emergence application of non-selective herbicides in hybrids for which resistance genes have been inserted. Two different glyphosate-resistant events, GA21 and NK603, are commercially available in maize. Both the events were released for commercial production in the United States in 1998 and 2001 respectively [53]. The first commercial glyphosate-tolerance event to be transformed into maize plants was GA21, which was commercialized during 1998 in USA and Canada in 1999. GA21 maize contains the modified (maize EPSPS) coding sequence (chloroplast transit peptide sequences from *Helianthus annuus* and the RuBisCo gene from *Zea may).* Effectively, this maize contains a modified version of its own EPSPS gene that could tolerate glyphosate and produce aromatic amino acids for protein production.

The second generation of glyphosate-tolerant maize event NK 603 was produced by two copies of the *cp4 epsps* gene was introduced into the maize genome to produce Roundup Ready corn event NK603. The *cp4 epsps* gene derived from the common soil bacterium *Agrobacterium* sp. strain *CP4* encodes for the naturally glyphosate tolerant EPSPS protein. NK603 has high tolerance to recommended field application rates of glyphosate, and the transgenic insertion neither created nor was linked to negative parameters that could affect human and animal health, the environment or yield performance. NK603 was first marketed in 2001 in both the USA and Canada and it has been commercialized in an increasing number of countries in the tropic regions including South Africa, Argentina, Philippines and Honduras. [54].

Efficacy of glyphosate tolerant maize

Regarding glyphosate efficacy of transgenic maize, [55] verified that transgenic maize showed substantially greater resistance to glyphosate applied as Roundup than the corresponding untransformed parental control. Treatment with 20 µg glyphosate applied as droplets to the second leaf was sufficient to produce complete kill in the parental controls, while 786 µg glyphosate had no effect upon survival of the transgenic plants. Equivalent field application rates for these dosages would be around 0.1 and 4kg (a.e.) ha^{-1} respectively. Reduction in foliage fresh weight was a more sensitive indicator of a given glyphosate treatment than mortality in both types of maize. Both methods for assessing herbicide efficacy indicated the transgenic plants to have more than 100 fold glyphosate resistance than the parental controls. [56] Indicated that no significant differences observed between herbicide tolerant corn event NK 603 and conventional maize hybrids. This was confirmed by evaluation of the feed performance in broiler chickens and a rat feeding study, included clinical and histological evaluations. The same study indicated that the environmental impact of Roundup Ready corn is comparable to conventional corn. Finally, the results of all these studies demonstrate that corn event NK603 is comparable to traditional corn with respect to food, feed and environmental safety.

In experimental fields at Ontario, no visual injury was observed in glyphosate tolerant maize crop by POE of glyphosate [57]. Similarly another study revealed that there was no adverse effects on plant establishment, plant height, maturity, vigour, yield or quality in glyphosate resistant cotton plants [58].

Effect of POE of glyphosate on glyphosate tolerant maize growth, yield and quality

Tallest maize plants (237.5 to 240.2 cm) were found in the plots that received glyphosate treatment singly at the three leaf stage of maize growth or repeated either at the seventh or twelfth leaf stage [59]. Grain yield (9135 kg ha^{-1}) was recorded under this treatment was comparable with weed free treatment, while unweeded control plots recorded 38.3 per cent lesser.

In the early maize planting at South Charleston, weed control with single application at the 5 cm weed height not exceed 62 per cent, but yield was not reduced compared with the weed free condition. The same study suggested that the application of glyphosate at 23 cm weed height resulted in a 22 and 15 per cent yield reduction in the early and late plantings, respectively, compared with weed free control. [60]. [61] concluded that, sequential applications of glyphosate in maize crop did not increase the grain yield. [62]. (2006) reported that early post treatment (EPOST) at three to four leaf stage of maize growth and weed-free treatment had similar results in silking date, dry matter accumulation, leaf area index in silking date, kernel per plant and grain yield. [63] witnessed that application of glyphosate as late post emergence and atrazine plus S-metalolachlor followed by glyphosate as post emergence or late post emergence produced higher grain yield (4790 kg ha^{-1}) compared to lowest grain yield with unweeded control and glyphosate alone as early post emergence. [64] found that both the conventional and glyphosate tolerant maize hybrids have high starch contents (729 g kg^{-1} and 736 g kg^{-1} of dry matter) and thus also a high nitrogen free extract content. The fat and crude protein levels reached 40 g kg^{-1} and 97 g kg^{-1} of dry matter respectively. [65] who compared the amino acid levels in genetically modified glyphosate-tolerant soybean also indicated the differences to be non-significant. These results of [66] indicated that the levels of proximate components (protein, ash and carbohydrate), fiber and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, and zinc) in the grain and forage of herbicide resistant corn event NK603 were comparable to those in the grain and forage of the non-transgenic control. In addition, these values were either within the published literature ranges. The content of the 18 amino acids measured in the grain of corn event NK603 was comparable to that in the grain of the non-transgenic control. The values for components in corn event NK603 all fell within the range of natural variability found in non-transgenic corn hybrids.

Conclusion

Post-emergence weed management in glyphosate tolerant maize is a promising alternative option for productivity enhancement in maize. However, future needs to develop of multi herbicide tolerant maize cultivars for effective control of all weeds including problematic weeds with single time application.

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Publication History

Received	10th Oct 2017
Revised	28th Oct 2017
Accepted	09th Nov 2017
Online	30th Nov 2017