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EFFECTS OF GLYPHOSATE POTASSIUM 660 g L^{-1} ON TRANSGENIC AND CONVENTIONAL CORN VARIETIES

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ABSTRACT

The transgenic corn variety, NK 603, which contains a gene called CP4 EPSPS (5-enolpyruvyl shikimate-3-phosphate synthase) which makes the corn plants tolerant to the glyphosate herbicide, on the other hand conventional corn varieties are sensitive to glyphosate. It was required to test the chlorosis and burn-down effect both in transgenic and conventional corn varieties due to glyphosate. The experiment was conducted to quantify the burn-down effects and chlorosis due to the application of herbicide glyphosate potassium 660 g L^{-1} on two varieties of corn at the Agriculture Faculty Research Station of Padjadjaran University, West Java, Indonesia, from December 2015 to April 2016. The experiment was arranged in randomized block design with 20 treatments and each treatment replicated twice. At 15 days and 20 days after planting, five transgenic corn varieties (C7 RR, 979 RR, 77 RR, 85 RR and 95 RR) and five conventional corn varieties (C7, 979, 77, 85 and 95) were applied with herbicide glyphosate potassium 660 g L^{-1} at a dose of 2 L ha^{-1}. The results showed that the herbicide was effective to control the weed in both transgenic and conventional corn varieties. The transgenic corn variety exhibited smaller percentages of chlorosis (0-20%) and no burn-down effect was observed following applications of glyphosate potassium 660 g L^{-1}. On the other hand, chlorosis and burn-down effects were found on all conventional corn varieties. The yield of transgenic corn varieties was higher than those of the conventional corn varieties.

Keywords: burn-down, chlorosis, conventional corn, potassium glyphosate, transgenic corn

INTRODUCTION

In Indonesia, corn (*Zea mays* L.), a subordinate crop to rice, is mainly processed as food for human, poultry and livestock. In 2016, the national demand for corn was 13.8 million tons. The national corn production has reached an all-time high among the national needs however, its demand cannot be fulfilled due to the low yield of corn. The presence of weed is one significant factor. Weed can reduce crop yields through competition for nutrients, water, space, and sunlight. Potential yield loss caused by weed competition, specifically in corn, is estimated to range between 16 to 80% (Paller 2002). The yield loss in corn caused by weed can reach 95% (Clay & Aguilar 1998), therefore the presence of weed in corn must be controlled. The use of herbicide is an economically important weed control method for corn, however, it should be applied carefully to avoid negative repercussions (Culpepper & York 2000). Transgenic corn varieties, as a result of genetic engineering, are known to increase the yield of corn (Dill 2005).

The corn variety, NK 603, is a new transgenic crop variety in Indonesia which contains a gene called CP4 EPSPS (5-enolpyruvyl shikimate-3-phosphate synthase) which makes the corn plants tolerant to the glyphosate herbicide. The CP4 EPSPS genes are the result of the isolation of soil bacterium *Agrobacterium tumefaciens* strain CP4 (Riches & Valverde 2002). Glyphosate is effective in controlling grass and broadleaf weeds. Foliar application of glyphosate is subjected to translocation to all parts of plant, whereas root absorption is negligible as the herbicide quickly decomposes in the soil (Klingman *et al.* 1975). The poisoning symptoms, due to glyphosate,

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develop slowly and are noticeable at 1-3 weeks after application. The leaf chlorosis and purpling veins, followed by necrosis and abnormality symptoms such as visible white spots and stripes are all symptoms of poisoning caused by glyphosate (Ashton & Crafts 1981). Leaf chlorosis is caused by the inhibition of the enzyme 5-enolpyruvil-shikimic-3-phosphate synthase (EPSP Synthase), which plays an important role in amino acid biosynthesis of phenylalanine, tyrosine, and tryptophan). Weeds diminish slowly in 1-2 weeks after the application of glyphosate, and then turn to brown leading to death (Monaco *et al.* 2002). The drift of glyphosate application on crop can be morphological defects, chlorosis, sterility, and yield loss (Heck *et al.* 2005).

Little is known of the effect of glyphosate potassium herbicide on the resistant NK603 and conventional corn varieties. Therefore, the present study aims to determine the efficacy of glyphosate potassium for weed control and to evaluate the chlorosis and burn-down effects of glyphosate potassium on NK603 transgenic and conventional corn varieties.

MATERIALS AND METHODS

The experiment was conducted at the Agriculture Faculty Research Station of Padjadjaran University, Jatinangor Sumedang, West Java, Indonesia from December 2015 to April 2016. Five corn transgenic varieties (C7 RR, 979 RR, 77 RR, 85 RR, and 95 RR), five conventional corn varieties (C7, 979, 77, 85, and 95), and fertilizers (urea, phosphate, KCl), fungicides with the active ingredient Dimeto-morf and Pyraclostrobin+ Epoxiconazole were used in this experiment. The insecticides used were Deltamethrin, Firpronil, and Betasiflutrin. The glyphosate potassium was applied by using semi-automatic knapsack sprayer with a pressure of 1 kg/cm^3. The experimental design used was randomized block design, which consisted of 20 treatments and each treatment was replicated two times; so that there were 40 plots (Table 1). The individual plot size was 3 m x 2.8 m. Glyphosate potassium herbicide was applied at 15 and 20 days after planting with the recommended dose of 2 L ha^{-1}.

Table 1 Application of glyphosate potassium herbicide at two different times to transgenic and conventional corn varieties

Symbol	Corn varieties	Time of application (DAP)*
A	Transgenic C7 RR	15
B	Conventional C7	15
C	Transgenic 979 RR	15
D	Conventional 979	15
E	Transgenic 77 RR	15
F	Conventional 77	15
G	Transgenic 85 RR	15
H	Conventional 85	15
I	Transgenic 95 RR	15
J	Conventional 95	15
K	Transgenic C7 RR	20
L	Conventional C7	20
M	Transgenic 979 RR	20
N	Conventional 979	20
O	Transgenic 77 RR	20
P	Conventional 77	20
Q	Transgenic 85 RR	20
R	Conventional 85	20
S	Transgenic 95 RR	20
T	Conventional 95	20

Note: *DAP = Day After Planting

The parameters observed were: 1) Weed dry weight (g) measured at 3 and 6 weeks after herbicide application, 2) Glyphosate injury in corn evaluated as chlorosis symptom at 3 and 5 days after herbicide application and burn-down symptom at 7 and 14 days after herbicide application (the symptoms of burn-down were assessed by using the score system: 0 = No burn-down, 0-5%, the shape or color of the young leaves are not normal; 1 = Mild burn-down, 6-20%, the shape or color of young leaves are not normal; 2 = Moderate burn-down, 21-50%, the shape or color of young leaves are not normal; 3 = Severe burn-down, 51-75%, the shape or color of young leaves are not normal; 4 = very severe burn-down, > 75%, the shape or color of young leaves are not normal to dry out and fall off until the plants die), 3) dry seed weight per plot (kg). All data were analyzed using the Minitab statistical Program.

RESULTS AND DISCUSSION

Vegetation Analysis before Applying Glyphosate Potassium Herbicide

The dominant weed species that were found in the field before spraying the glyphosate potassium herbicide were the three broadleaved species of *Bidens pilosa* L., *Cleome rutidosperma* DC. and *Alternanthera sessili* (L) R.Br. ex DC.

Weed Dry Weight

Table 2 and Table 3 show the effect of timing application of glyphosate potassium herbicide 660 g L^{-1} on the transgenic and conventional corn varieties at 3 and 6 weeks after herbicide applications. A total of 20 weed species, comprising of 14 broadleaves, 5 grasses, and one species of sedge were observed in both transgenic and conventional corn fields. No significant difference was observed in the dry weight of the predominant weeds (*Bidens pilosa*, *Cleome rutidosperma* and *Alternanthera sessilis*), dry weight of other species of weeds, and dry weight of weed in total at both application times. These results indicate that the application of glyphosate potassium herbicide 660 g L^{-1}, at 15 and 20 days after planting, has similar effectiveness in controlling all weed species, regardless of their composition and dominance in the field. Other recorded weeds species were *Cyperus rotundus* L, *Commelina diffusa* Burm. f, *Mimosa invisa* Mart. ex Colla, *Panicum repens* L, *Borreria alata* (Aubl.) DC, *Mimosa pudica* L, *Phyllanthus niruri* Linn, *Phyllanthus urinaria* L, *Synedrella nodiflora* (L) Gaertn, *Emilia sonchifolia* (L.) DC., *Digitaria ciliaris* (Retz) Koel, *Ageratum conyzoides* L, *Oxalis barrelier* Li, *Axonopus compressus* (Swartz.) Beauv, *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L, and *Imperata cylindrica* (L.) Raeusch.

Table 2 Dry weights of the dominant weeds

Symbol	Variety	Treatment DAP (days)**	Weed dry weight of *Bidens pilosa* (g/0.25 m²)		Weed dry weight of *Cleome rutidosperma* (g/0.25 m²)		Weed dry weight of *Alternanthera sessilis* (g/0.25 m²)	
			3 WAA***	6 WAA	3 WAA	6 WAA	3 WAA	6 WAA
A	C7 RR	15	0.20 a*	7.05 a	0.20 a	0.00 a	0.10 a	0.85 a
B	C7	15	0.30 a	0.00 a	0.30 a	0.00 a	0.40 a	4.85 a
C	979 RR	15	0.00 a	0.40 a	0.00 a	0.00 a	0.00 a	3.10 a
D	979	15	0.00 a	0.00 a	0.00 a	0.10 a	0.00 a	0.90 a
E	77 RR	15	0.35 a	2.15 a	0.35 a	0.00 a	0.30 a	0.55 a
F	77	15	0.00 a	1.60 a	0.00 a	0.10 a	0.00 a	1.70 a
G	85 RR	15	0.05 a	0.05 a	0.05 a	0.00 a	0.05 a	0.85 a
H	85	15	0.00 a	0.20 a	0.00 a	0.00 a	0.00 a	2.80 a
I	95 RR	15	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.80 a
J	99	15	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	6.40 a
K	C7 RR	20	0.50 a	0.00 a	0.50 a	0.00 a	0.60 a	1.10 a
L	C7	20	0.80 a	0.00 a	0.80 a	0.25 a	0.80 a	3.35 a
M	979 RR	20	0.05 a	0.20 a	0.05 a	0.00 a	0.05 a	0.55 a
N	979	20	0.30 a	0.40 a	0.30 a	0.00 a	0.30 a	1.25 a
O	77 RR	20	0.50 a	0.00 a	0.50 a	0.00 a	0.45 a	0.10 a
P	77	20	0.15 a	0.00 a	0.15 a	0.00 a	0.05 a	5.50 a
Q	85 RR	20	0.15 a	0.25 a	0.15 a	0.00 a	0.15 a	0.00 a

R	85	20	0.40 a	0.00 a	0.40 a	0.00 a	0.40 a	1.65 a
S	95 RR	20	0.30 a	0.10 a	0.30 a	0.00 a	0.45 a	1.20 a
T	99	20	0.85 a	3.60 a	0.85 a	0.00 a	0.95 a	0.60 a

Notes: *Means with the same superscript in a column do not significantly differ based on the Scott-Knott test at P<0.05
**DAP: Day After Planting
***WAA: Week After herbicide Application

Table 3 Dry weight of other weed species and weed total dry weight

Symbol	Variety	Treatment DAP (days)**	Weed total dry weight ($g/0.25\ m^2$)		Dry weight of other weed species ($g/0.25\ m^2$)	
			3 WAA	6 WAA	3 WAA	6 WAA
A	C7 RR	15	0.30 a*	1.05 a	0.00 b	0.20 a
B	C7	15	0.70 a	3.05 b	0.00 b	0.15 a
C	979 RR	15	0.00 a	6.00 a	0.00 b	2.50 a
D	979	15	0.00 a	1.45 a	0.00 b	0.45 a
E	77 RR	15	1.30 a	2.70 a	0.00 b	0.00 a
F	77	15	0.00 a	4.20 a	0.00 b	0.80 a
G	85 RR	15	0.20 a	1.05 a	0.00 b	0.15 a
H	85	15	1.55 a	6.75 a	0.80 a	1.47 a
I	95 RR	15	0.00 a	1.20 a	0.00 b	0.40 a
J	99	15	0.00 a	6.85 a	0.00 b	0.45 a
K	C7 RR	20	1.55 a	1.40 a	0.00 b	0.30 a
L	C7	20	1.90 a	4.25 a	0.00 b	0.65 a
M	979 RR	20	0.20 a	1.15 a	0.00 b	0.40 a
N	979	20	0.80 a	5.20 a	0.00 b	3.55 a
O	77 RR	20	1.20 a	0.40 a	0.00 b	0.30 a
P	77	20	0.40 a	7.35 a	0.00 b	1.85 a
Q	85 RR	20	0.30 a	1.75 a	0.00 b	1.50 a
R	85	20	1.35 a	2.20 a	0.00 b	0.55 a
S	95 RR	20	3.50 a	2.25 a	0.00 b	0.95 a
T	99	20	0.00 a	6.35 a	0.00 b	3.15 a

Note: *Means with the same superscript in a column do not significantly differ based on the Scott-Knott test at P<0.05.
**DAP: Day After Planting
***WAA: Week After herbicide Application

Chlorosis, Burn-down and Yield of Corn

At 3 and 5 days after herbicide application, chlorosis was observed on corn as result of herbicide phytotoxicity (Table 4). The percentage of chlorosis in conventional corn varieties was 60-85%, whereas the percentage in transgenic corn varieties was much lower at 0-20%. All the conventional corn varieties exhibited higher rate of chlorosis as compared to transgenic corn varieties at 3 and 5 days after herbicide application. This is probably because the conventional corn plants do not have the CP4 EPSPS genes that make corn plants resistant to spraying glyphosate potassium herbicides (Table 4). Glyphosate is easily absorbed by leaves and is translocate able in plants, moving through the symplastic system (Roberts 1982). Glyphosate kills plants by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is a key enzyme in the shikimate biosynthetic pathway which is necessary for the production of the aromatic amino acids, auxin, phytoalexins, folic acid, lignin, plastoquinones and many other secondary products.

The application of glyphosate potassium at 15 and 20 days after planting showed significantly more severe burn-down symptoms in conventional corn varieties than in transgenic corn varieties at 7 and 14 days after herbicide application (Table 1; Fig. 1-6). The burn-down score in the conventional corn species is 4 (very severe burn-down), whereas in the transgenic corn species, the score is 0 (no burn-down). It was also observed that all conventional corn varieties were totally killed by the application of glyphosate potassium herbicide at 15 and 20 days after planting. The burn-down symptoms in corn plants were clearly evident on the young leaves. The observable symptoms were, firstly all the young leaves turned yellowish-brown, followed by stunted growth, and finally death. The application of glyphosate potassium herbicide at 15 and 20 days after planting produced only a small percentage of chlorosis (0-20%), with no visible burn-down effect on the transgenic corn varieties. This is probably because the transgenic corn varieties have been inserted with the CP4 EPSPS gene derived from *Agrobacterium tumefaciens* that is insensitive to glyphosate. The transgenic corn is tolerant to glyphosate (Heck *et al.* 2005). In contrast, conventional corn varieties are sensitive to glyphosate and therefore, the use of glyphosate for weed control in non-transgenic corn field is not suggested.

The yield of transgenic corn varieties that were sprayed by glyphosate potassium herbicide 660 g L^{-1} was between 7.13 to 10.57 ton ha^{-1}. On the contrary, the yield of conventional corn varieties was significantly lower at 0-2.6 ton ha^{-1} (Table 4).

Table 4 Chlorosis, burn-down and yield of corn

Symbol	Variety	Treatment DAP (days)**	Chlorosis (%)		Burn-down		Yield of Corn
			3 DAA***	5 DAA	7 DAA	14 DAA	Ton Ha^{-1}
A	C7 RR	15	0	10	0.00 b*	0.00 b	10.57 a
B	C7	15	85	85	4.00 a	4.00 a	0.00 c
C	979 RR	15	0	0	0.00 b	0.00 b	9.54 a
D	979	15	75	85	4.00 a	4.00 a	0.00 c
E	77 RR	15	0	5	0.00 b	0.00 b	9.02 a
F	77	15	85	85	4.00 a	4.00 a	0.00 c
G	85 RR	15	0	5	0.00 b	0.00 b	8.60 a
H	85	15	60	85	4.00 a	4.00 a	0.00 c
I	95 RR	15	0	5	0.00 b	0.00 b	8.14 a
J	99	15	60	85	4.00 a	4.00 a	0.00 c
K	C7 RR	20	0	0	0.00 b	0.00 b	9.56 a
L	C7	20	75	85	4.00 a	4.00 a	2.60 b
M	979 RR	20	0	0	0.00 b	0.00 b	10.68 a
N	979	20	60	85	4.00 a	4.00 a	1.10 b
O	77 RR	20	0	0	0.00 b	0.00 b	8.67 a
P	77	20	75	85	4.00 a	4.00 a	1.15 b
Q	85 RR	20	5	20	0.00 b	0.00 b	7.13 a
R	85	20	60	85	4.00 a	4.00 a	1.67 b
S	95 RR	20	0	0	0.00 b	0.00 b	9.30 a
T	99	20	60	60	4.00 a	4.00 a	2.04 b

Notes: *Means with the same superscript in a column, do not significantly differ according to the Scott-Knott test at $P<0.05$
**DAP: Day After Planting
***DAA: Day After herbicide Application

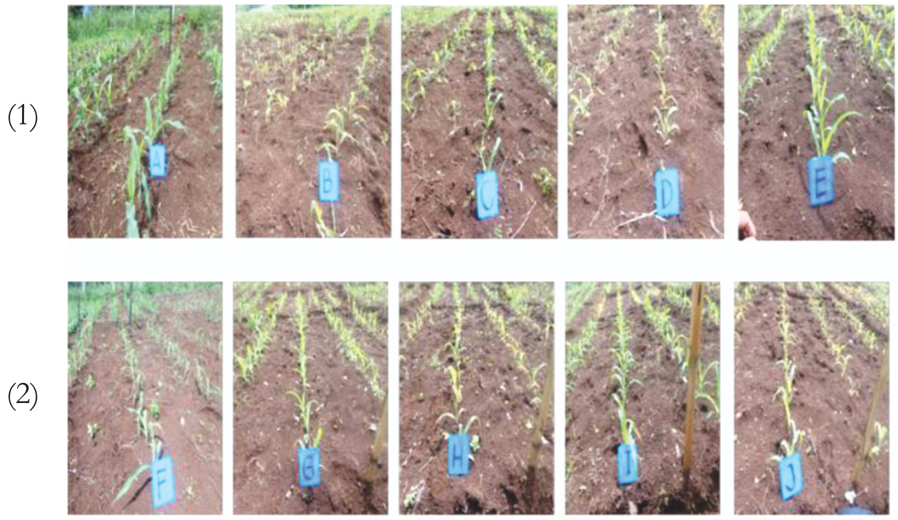

Figure 1 Observed chlorosis: (1) at 3 days after application of herbicide on transgenic and (2) at 15 days after planting conventional varieties sprayed with glyphosate potassium herbicide

Figure 2 Observed chlorosis: (1) at 5 days after application of herbicide on transgenic and (2) at 15 days after planting of conventional varieties sprayed with glyphosate potassium herbicide

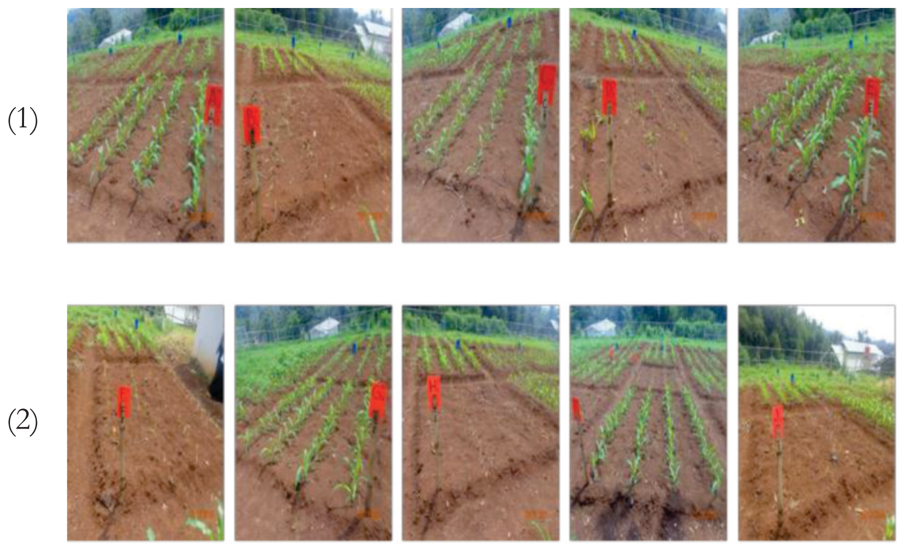

Figure 3 Observed burn-down effects: (1) at 7 days after application of herbicide on transgenic and (2) at 15 days after planting of conventional varieties sprayed with glyphosate potassium herbicide

Figure 4 Observed burn-down effect: (1) at 14 days after application of herbicide on transgenic and (2) at 15 days after planting of conventional varieties sprayed with glyphosate potassium herbicide

Figure 5 Observed burn-down effects: (1) at 7 days after application of herbicide on transgenic varieties and (2) at 20 days after planting of conventional varieties sprayed with glyphosate potassium herbicide

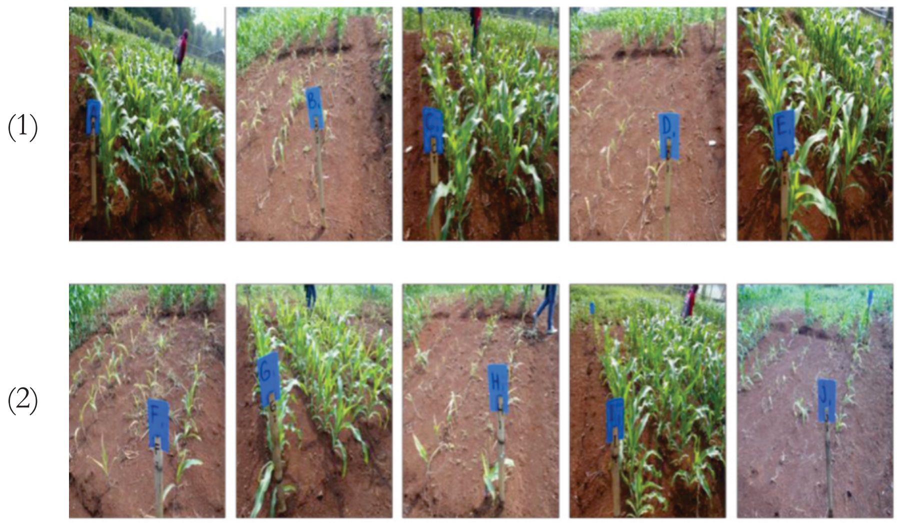

Figure 6 Observed burn-down effects: (1) at 14 days after application of herbicide on transgenic and (2) at 20 days after planting of conventional varieties sprayed with glyphosate potassium herbicide

CONCLUSION

All the transgenic corn varieties exhibited small percentages of chlorosis (0-20%) and no burn-down effects following the application of glyphosate potassium herbicide. Contrary to the transgenic varieties, all the conventional corn varieties displayed severe chlorosis and burn-down effects. Moreover, better weed control was evident at the glyphosate potassium application time of 20 days after planting in both transgenic and conventional corn varieties.

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