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Application of herbicides on parental lines (A clearfield® and R) of hybrid rice at post-flowering stage for production

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Abstract

The hypothesis of this study is based on the fact that parental line A-Clearfield® (male-sterile genotype) (Clearfield provide has tolerance to imidazolinone herbicides) and line R (male-fertile genotype - pollinator) is sensitive to the herbicide. So after flowering, we may apply an imidazolinone herbicide (Kifix®) to the plantings to kill line R, and harvesting only the hybrid seeds. The objective was to test the use of imidazolinone herbicide on parental lines (A-Clearfield® which is resistant to Kifix herbicide) and R no Clearfield® (non-resistant to Kifix genotype) as a strategy to improve production of hybrid seed in rice. Two trials were conducted to evaluate (A) the use of doses of imidazolinone herbicide in line R (plant control, number of grains per panicles, grain yield and seed germination; and (B) the use of different doses of imidazolinone herbicide in line A Clearfield® grain yield and seed germination. Trials were conducted in the tropical region of Brazil in randomized complete block design in factorial scheme 5 x 2 x 2. The treatments were the combination of five Kifix (Imazapyr, 525 g kg^{-1} + Imazapic, 175 g kg^{-1}) rates applied on two no Clearfield® rice genotypes (first trial) or Clearfield® rice genotypes (second trial) in two growing seasons. The results showed application of herbicide doses higher than 150 g ha^{-1} after full flowering stage can cause reduction in growth and development of no Clearfield® rice. The use of Kifix herbicide in CL (Clearfield®) rice cultivars did not affect the grain yield, mass of 1000 grains, seed germination, first count germination, seedling emergence and seedling length. New researches should be done to define plant arrangement suitable for line R (no Clearfield®) and line A Clearfield® to find the optimum condition to produce hybrid seeds. From our results, we could see that the use of parental line A Clearfield® with a line R no Clearfield®, after flowering, provided the production of only hybrid seeds in the area, without reduction in seed quality.

Keywords: *Oryza sativa*, agronomic strategies, Kifix, imidazolinone.
Abbreviation: CL_Clearfield; ALAM_Asociation Latinoamericana de Malezas.

Introduction

Rice is a staple food for more than half of world population (Tesio et al., 2014; Nascente et al., 2013; Prasad, 2011). There is an increasing demand of rice worldwide. It is expected that more than 116 million additional metric tons will be required in the year 2035 compared with the world rice production in 2010 (GRiSP, 2013). Therefore, the development of technologies that result in a higher rice grain yield is necessary to meet this demand and feed the world (Akhter et al., 2007; Qin et al., 2013). Globally and especially in Asian countries, the use of hybrid rice has achieved a significant increase in the grain yield compared with the use of traditional cultivars (Tan et al., 2002). According to Ravi et al. (2007), Krishnakumar et al. (2005) and Virmani (2003) in countries such as China and India, the use of hybrid rice raised the average grain yield by 15-30% compared with traditional methods. These countries are the world's largest producers of rice and their cultivation of hybrid rice is widespread and has been used by farmers for more than 20 years (Kim et al., 2007; Mondo et al., 2016). These increases in rice grain yield represent, on average, an increase in productivity from 10,000 kg to 13,000 kg ha^{-1}.
To enable the production of hybrid rice seeds, it is important to have cytoplasmic male sterility, which allows outcrossing between the different plants. It is also vital to have a genetic system that restores male fertility (restorer) (Shahid et al., 2013; Huang et al., 2013; Tesio et al., 2014). The production of hybrid rice seeds requires a system composed of three lines: male-sterile (line A), male-fertile with the ability to maintain sterility of the line A (line B) and another, also male-fertile, with the restoring capacity for fertility in line A (line R). The combination of the first two lines (A and B) produces seeds that originate from male-sterile plants (line A seeds). The cross between A and R lines produces hybrid seeds originating from fertile plants (Bragantini et al., 2001).

For a successful seed production, A and R lines must be sown to ensure the synchronization of the flowering of lines, encouraging cross-pollination, which is a crucial factor in the production of hybrid seeds (Mao et al., 1998). However, after pollination the line R becomes a problem for the farmers, once it can mix with the rice hybrid seeds and contaminates the seed set. Therefore, development of technologies that allow the pollination of line A by line R and after this eliminate line R before harvesting line A facilitate high quality rice hybrid seeds production (no mixture) with high speed in the harvesting operation.

Clearfield® rice could be an opportunity that selectively controls line R (no Clearfield) with imidazolinone herbicides, when using a line A Clearfield®. Imidazolinones herbicides include the active groups imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin (Tan et al., 2005). These herbicides, even in low rates, have a toxic effect on dicotyledon species and on grasses and cyperaceous plants (Rangel et al., 2010). To generate imidazolinone resistant rice lines, rice seeds were treated with the mutagen ethyl methanesulfonate and the resistant rice genotypes produced (Sudianto et al., 2013). Imidazolinone herbicides act as non-competitive inhibitors of the ALS enzyme and halt the production of these amino acids. Without these amino acids, plants will slowly die due to their inability to synthesize proteins, which are important for cell division. The mutant ALS gene in Clearfield® rice makes it insensitive to imidazolinones herbicides (Rangel et al., 2010).

The hypothesis of this research is using parental line A Clearfield® (Clearfield provide tolerance to the action of imidazolinone herbicides for rice plants) with a line R (pollinator) which is sensitive to the herbicide, after flowering. We applied the imidazolinone herbicide in total area and killed line R. After that, we only harvested hybrid seeds, which were produced on the line A. This research had the objective to test the use of imidazolinone herbicide on production of hybrid rice.

Results

Trial 1 – Application of rates of Kifix herbicide on non-resistant rice plants

Rice plants without resistance to the Kifix herbicide (no-Clearfield) was not affected by growing season and genotype (Table 1). The grading scale to determine the effect of herbicides on plants ranged from 1 (no effect on rice plants-control) to 5 (rice plants died), which showed 2.65. In other words, application of the herbicide after rice flowering stage was not able to kill all the rice plants.

The herbicide rates significantly affected rice plants (Fig 1). There were not interactions among the evaluated factors. Grading scale of controlled plants from 1 (no rice plants controlled by the Kifix herbicide) to 5 (all rice plants controlled by the Kifix herbicide) fitted to a linear regression with increased values as increased rates of herbicide. In this sense, to control 80% of rice plants, a rate of 400 g ha^{-1} of the herbicide was necessary, a value four times bigger than the recommended rate (100 g ha^{-1}).

Number of grains per panicle, mass of 1000 grains, percentage of germination and first count of germination were affected by herbicide rates (Fig 2). All of these four variables had the data fitted to a quadratic regression and values were decreased due to increased rates of herbicide. Therefore, despite the application of herbicide in no resistant rice plants to this herbicide, its application provided significant reduction in number of grains per panicle, mass of 1000 grains, percentage of germination and first count of germination of the remain rice plants.

Trial 2 – Application of rates of Kifix herbicide in resistant rice plants

The growing season affected all variables except for seedling emergence (Table 2). Therefore, grain yield, mass of 1000 grains, rate of seed germination, first count of germination and seedling length had higher values in the growing season 2013/14 than in the growing season 2012/13.

Genotypes affected grain yield, mass of 1000 grains, seed germination and seedling emergence (Table 2). Primavera CL had higher grain yield, mass of 1000 grains, seed germination and seedling emergence than Puitá INTA CL.

Regarding herbicide rates, rice grain yield, mass of 1000 grains and seed viability was not affected (Table 2). Therefore, applying herbicide in the rates from 50 to 400 g ha^{-1} at the beginning of rice development does not affect rice development and rice seeds quality.

Discussion

Controlling non-resistance rice plants after pollination

The application of increasing rates of herbicide (Kifix) was efficient to control non-resistance (to herbicide) rice plants (Fig 1). However, the recommended rate (100 g ha^{-1}) (Rangel et al., 2010) was not enough to control rice plants. Only rates of 400 g ha^{-1} was able to control more than 80% of rice plants. The increase of Kifix herbicide rates results in increasing phytotoxicity of rice plants (Dal Magro et al., 2006). The need to increase rates of Kifix could be because the recommendation is to apply the herbicide when weeds or target plants are at 2 to 4 leave stage (BASF, 2017). However, in our trial we had to apply the herbicide after rice pollination, once we tried to pollinate the rice plants first (parental line A) and then kill male plants (parental line R) to have only hybrid seeds at the harvesting time. However, increasing rates of herbicide was able to reduce mass of 1000 grains and number of grains per panicle of the remained rice plants. Rate of 150 g ha^{-1} of herbicide was enough to kill seeds and avoid rice seed germination. Therefore, planting parental lines R and A (no Clearfield, non-resistance to Kifix herbicide), we had to increased rates of the herbicide more than recommended, once it should be applied latter in the rice development. We could see that the technique to apply Kifix was effective to control parental line R after pollination.

Effect of Kifix herbicides on cultivars and growing seasons

Primaver CL had higher grain yield than Puitá INTA CL, probably because it is a cultivar released for tropical region, like the place where trials were performed and Puitá INTA CL is a cultivar for subtropical conditions (Santos et al., 2006). Growing season 2013/2014 was more productive than growing season 2012/2013, probably because in the growing

Table 1. Grading scale from 1 (no rice plant was controlled; intact plant) to 5 (rice plant died) of rice plants due to the application of Herbicide Kifix (Imidazolinone active ingredient) after rice flowering as a function of growing season and genotypes, and ANOVA (F probability). Santo Antônio de Goiás, Brazil.

Factors	Rice Plant controlled by Kifix herbicide
Growing season	Grading scale
2012/2013	2.65
2013/2014	2.65
Genotype	
L106R	2.63
CIRAD 450A	2.68
Factors	ANOVA - F probability
Growing season (GS)	0.9999
Genotype (G)	0.6707
Herbicide rates (HR)	<0.001
GS x G	0.0613
GS x HR	0.0534
G x HR	0.0710
GS x G x HR	0.2107

The model used for statistical analysis was the factorial design (factors were herbicide rates, genotypes and growing seasons). Qualitative data were analyzed by comparing the means using LSD test, and statistical significance was defined as p ≤ 0.05.

Table 2. Grain yield (GY), mass of 1000 grains (1000G), rate of seed germination (SG), first count of germination (FCG) and seedling emergence (SE) and seedling length (SL) as a function of herbicide application (Kifix), growing season and genotypes. Santo Antônio de Goiás, Brazil.

Factors	GY	1000G	SG	FCG	SE	SL
Growing season	kg ha^{-1}	g	%	%	%	cm
2012/2013	3104 b	20.10 b	70.28 b	51.80 b	59.33	7.93 b
2013/2014	4729 a	23.16 a	78.03 a	58.55 a	57.53	12.32 a
Genotype						
Primavera CL	7302 a	23.76 a	79.90 a	57.00	68.00 a	8.95
INTA Puitá CL	532 b	21.50 b	68.29 b	53.35	48.76 b	9.62
Herbicide rates						
0	5289	22.23	74.00	54.71	58.83	10.27
50	4706	22.37	71.96	55.71	53.50	9.27
100	5308	22.30	75.04	57.88	56.92	9.35
200	5116	22.15	69.50	51.88	54.67	8.69
400	4805	22.15	70.29	52.21	51.96	9.41
Factors	ANOVA F probability					
Growing season (GS)	<0.001	<0.001	0.0003	0.0009	0.4998	<0.001
Genotype (G)	<0.001	<0.001	<0.001	0.0620	<0.001	0.1282
Herbicide rates (HR)	0.5371	0.8007	0.1588	0.3194	0.3051	0.1707
GS x G	<0.001	<0.001	0.0003	0.0009	0.4998	<0.001
GS x HR	0.3056	0.7333	0.9029	0.9757	0.9479	0.8046
G x HR	0.5565	0.4627	0.3539	0.8071	0.6145	0.3370
GS x G x HR	0.3056	0.7333	0.9029	0.9757	0.9479	0.8046

The model used for statistical analysis was the factorial design (factors were herbicide rates, genotypes and growing seasons). Qualitative data were analyzed by comparing the means using LSD test, and statistical significance was defined as p ≤ 0.05.

Table 3. Chemical soil attributes in the experimental area at the layer 0-0.20 m in the 2012/2013 and 2013/2014 growing seasons. Santo Antônio de Goiás, Brazil.

Growing season	pH	SOM	K	Ca	Mg	Al	H+Al	P	Cu	Fe	Mn	Zn
	(water)	g dm^{-3}	-----------$cmol_c$ dm^{-3}-----------					--------------mg dm^{-3}-------------				
2012/13	5.4	36.8	0.15	1.6	1.0	0.2	3.0	6.1	1.1	15.3	8.9	4.5
2013/14	5.5	33.3	0.34	1.3	0.7	0.1	3.8	7.5	1.1	18.0	9.4	4.2

pH – hydrogen potential, SOM – soil organic matter, K – potassium, Ca – calcium, Mg – magnesium, Al – aluminum, P – phosphorus, B – boron, Fe – iron, Mn – manganese, Zn – zinc.

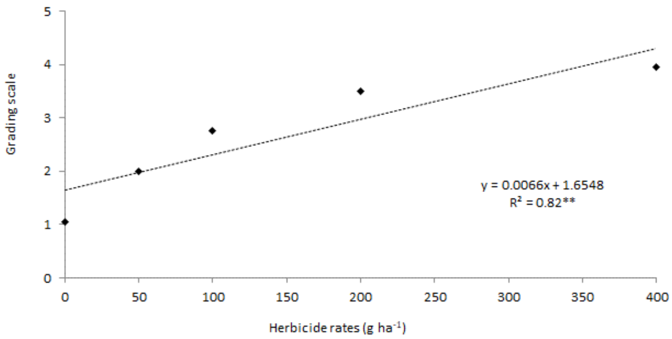

Fig 1. Grading scale from 1 (no rice plant was controlled; intact palnt) to 5 (rice plant died) of rice plants no-resistant to Kifix herbicide as a function of herbicide rates.

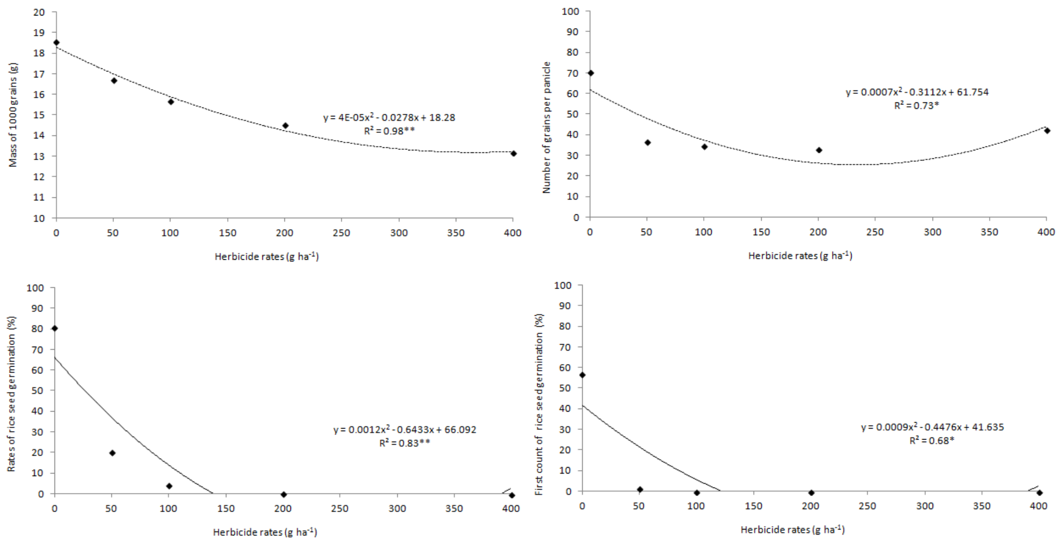

Fig 2. Number of grains per panicle, mass of 1000 grains, rate of seed germination and first count of germination of genotype L106R as a function of the rates of Kifix herbicide. Santo Antônio de Goiás, Brazil. Growing season 2013/2014.

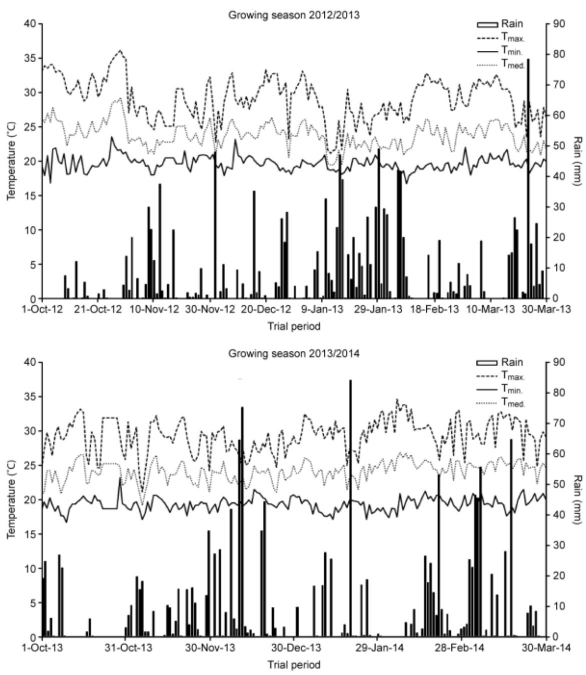

Fig 3. Average temperature and rain fall during the trials. Santo Antônio de Goiás, growing seasons 2012/13 e 2013/14.

season 2013/2014 there were more rains and the temperature was lower than growing season 2012/2013 (Fig. 1).

Effect of increasing rates of Kifix herbicide on resistant rice plants

Grain yield, mass of 1000 grains, seed germination, first count of germination, seedling emergence and seedling length was not affected by herbicide rates (Table 2). Therefore, as expected, the use of Kifix herbicide in genotypes CL, resistant to Kifix herbicide (Primavera CL and Puitá INTA CL) did not provide any problems in the variables evaluated, even in the rate 400 g ha^{-1}, which is four times greater than the recommended rate. Several authors have shown that rice development of CL plants was not affected by application of Kifix (Pellerin and Webster, 2004; Villa et al., 2006; Rangel et al., 2010).

In our trial, we had the hypothesis that using parental line A Clearfield® (resistant to Kifix herbicide) with a line R (pollinator) sensitive to the Kifix herbicide, we could apply the imidazolinone herbicide after flowering in total area and kill line R, and harvest only hybrid seeds, produced on line A. Therefore, according to our results, we can accept the hypothesis, apply Kifix herbicide in rates greater than 150 g ha^{-1} on line R no-Clearfield® (no resistance to the Kifix herbicide) after pollination, can lead to harvest only hybrid seeds from line A.

Our results could be very promising using line A Clearfield® and line R no Clearfield®. It allows increasing population of line A and better distribution of line R in the field. After pollination, we should use the herbicide Kifix and harvest only rice hybrid seeds. This could allow increasing rice hybrids seeds yields and reduce cost of production, allowing more farmers to use hybrids seeds. Trials should be done with the use of line R no Clearfield® and line A CL in the same place with the use of herbicide Kifix to evaluate the production of rice hybrid seeds, economic viability of this technology and the arrangement of plants line R and A CL in the field.

Materials and methods

Site description

The experiments were conducted at Fazenda Capivara, Embrapa Rice and Beans, located in Santo Antônio de Goiás, GO, at 16 ° 28'00 " S and 49 º 17'00 " W and 823 m of altitude. The climate is tropical savanna and considered Aw according to the Köppen classification. There are two well-defined, normally dry seasons from May to September (autumn/winter) and two rainy seasons from October to April (spring/summer). Temperature and rainfall data were monitored during the experiment (Fig 3).

The soil was classified as clay loam (kaolinitic and thermic Typic Haplorthox) acidic soil (Embrapa, 2006). Before the trials in October 2012 and October 2013, chemical analyses were performed at a depth of 0-0.20 m to characterize the experimental area (Table 3). The chemical analyses were performed according to the methodology proposed by Claessen (1997). The soil pH was determined in a 0.01 mol L^{-1} $CaCl_2$ suspension (1:2.5 soil/solution), and the exchangeable Ca, Mg, and Al were extracted with neutral 1 mol L^{-1} KCl in a 1:10 soil/solution ratio and determined by titration with a 0.025 mol L^{-1} NaOH solution. Phosphorus and exchangeable K were extracted with a Mehlich 1 extracting solution (0.05 M HCl in 0.0125 M H_2SO_4). The extracts were calorimetrically analyzed for P, and flame photometry was used to analyze K. The base saturation values were calculated using the results of the exchangeable bases and total acidity at pH 7.0 (H + Al). Micronutrients were determined in the Mehlich 1 extract by atomic absorption, and the organic matter was determined by the method of Walkley and Black.

Experimental design and treatments

Two independent experiments were performed in different areas for two consecutive growing seasons (2012/13 and 2013/14). The first trial was in randomized complete block design in factorial scheme 5 x 2 x 2. Treatments were the combination of five Kifix (Imazapyr, 525 g kg^{-1} + Imazapic, 175 g kg^{-1}) rates (0, 50, 100 (recommended rate), 200 and 400 g ha^{-1}) with two no-Clearfield® (no herbicide resistance) rice genotypes (L106R, line R and Cirad 450 A, line A) applied after rice pollination and two growing seasons. The goal was to see if after rice pollination, the herbicides effectively control rice plants.

The second trial was also in randomized complete block design in factorial scheme 5 x 2 x 2. Treatments were the combination of five Kifix rates [0, 50, 100 (recommended rate), 200 and 400 g ha^{-1}] applied during 2-4 leaves stage of weeds (Basf, 2017), with two potential lines A Clearfield (resistant to Kifix herbicide) in the production system of hybrid rice (Puitá INTA CL and Primavera CL) and two growing seasons. The goal here is to see if higher doses of Kifix herbicide could damage the hybrid seed production. The plots for both trials had a dimension of 1.75 m (five rice rows) x 5 m long. The usable area of the plots consisted of three central rows of rice, disregarding 0.50 m on each side.

Rice crop management

The experiments were sown on December 1st, 2012 and November 22nd, 2013. The average emergence of rice seedling took place five days after sowing. The row spacing was 0.35 m, and the seeding rate was 80 viable seeds per meter. The fertilization was carried out based on soil analysis (Sousa and Lobato, 2004). Therefore, we applied 110 kg ha^{-1} of P_2O_5 as triple superphosphate and 20 kg ha^{-1} of N as urea at sowing, supplemented with an additional 60 kg ha^{-1} of N as urea at topdressing 20 days after rice emergence. According to soil analysis, K was not needed. Cultural practices were performed according to standard recommendations for a rice crop to keep the area free from weeds, diseases and insects.

Evaluations of traits

Trial 1- Evaluation of the seed viability on line R and line A due to herbicide application after pollination

Rice plants control by the herbicide: A grading scale from 0% (no effect by the herbicide on plant meaning no control) to 100% (plant died by the herbicide) in the usable area at the harvesting time. We used the scale based on the Asociation Latinoamericana de Malezas - ALAM (1974).

Number of grains per panicle: Ten randomly panicles was sampled in the usable area and the number of grains per panicles in each panicle was counted and the average calculated.

Mass of 1000 grains: counting the number of grains in the panicles samples and estimating the weight of 1000 grains from each plot, corrected to 13% of water content.

Percentage of germination: four replications of 50 seeds per plot were rolled in germination paper towels. For each plot, seeds were uniformly distributed over two sheets of germination paper towels and covered with another sheet. The sheets were previously moistened with water ratio equals to 2.5 times the mass of dry paper. The rolls were vertically placed in germination chambers at 25° C in the dark. The percentage of normal seedlings was recorded on the 14th day after sowing.

Trial 2- Evaluation of plant performance and seed viability in CL genotypes

Grain yield: was determined by weighing the harvested grain of each plot, corrected to 13% of the water content and converted to kg ha^{-1}.

Mass of 1000 grains: like previously described in trial 1.
Germination: like previously described in trial 1.

Seedling length: four replications of 20 seeds per treatment were rolled in germination paper towels, similar to the germination test. To avoid contact between seedlings, seeds were positioned with radicle pointing downwards. In order to standardize root growth conditions for all samples, seeds were uniformly distributed along two lines of 10 seeds each, on the upper third of the paper towels. All seedlings were measured by total length (cm) seven days after sowing.

Seedling emergence: four replications of 50 seeds per treatment were uniformly distributed in plastic boxes (52 × 31 × 10 cm) filled with sand moistened to 70% of its water holding capacity, and maintained at room temperature (~25 °C). The emerged seedlings were counted daily until the 14th day after sowing. Emerged seedling was considered anyone with at least 2-cm plumule emerged from the substrate. The results were expressed as percentage of emerged seedlings.

Statistical analyses

The model used for statistical analysis was the factorial design for all experiments (factors were herbicide rates, genotypes and growing seasons). Qualitative data were analyzed by comparing the means using LSD test, and statistical significance was defined as $p \leq 0.05$. Quantitative data were analyzed by regression analysis. The statistical package SAS was used for statistical analysis.

Conclusion

Our results shows that rates greater than 150 g ha^{-1} of Kifix herbicide reduces development of no-Clearfield® rice when applied after full flowering stage, which could help to improve production of rice hybrid once after pollination. We were able to kill the pollinator and harvest only hybrid rice seeds. We could also see that the use of Kifix herbicide in CL rice cultivars did not affect grain yield, mass of 1000 grains, seed germination, first count germination, seedling emergence and seedling length.

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