Application of gibberellic acid (GA_3) in dosage for three hybrid rice seed production in the Philippines

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GA_3 application was very effective in increasing seed set rate and seed yield through elongation of plant height, promoting panicle and spikelet exsertion, enhancing stigma exsertion and longevity and receptivity. During the 2005 wet season in the Philippines, 225, 150 and 150 g ha^{-1} of GA_3 dosage were found the most effective and economical thus recommended for Mestizo 1, 2, 3 seed production, respectively.

Key words: Gibberelic acid (GA_3), Mestizo hybrid rice varieties

Introduction

Panicle poor exsertion from flag leaf sheath is a problem in cytoplasmic male sterile lines. Foliar application of gibberellic acid (GA_3) is an essential technique in promoting panicle exsertion and obtaining high cross-pollinated seed set in hybrid rice seed production (Duan and Ma, 1992), which was proven and a successful approach in significant in creased in seed yield in China. In hybrid rice seed production, GA_3 was also used to increase the duration of floret opening, the rate of stigma exsertion, lengthen the duration of stigma receptivity and adjust the plant height of both parents, and increase the growth rate of secondary and tertiary tillers so that they bear panicles (Virmani and Sharma, 1993). Hybrid rice seed producers in China use a very high dosage (150-300 g ha^{-1}) to get high seed yields (Virmani *et al.*, 2002). Yuan (1985) suggested that GA_3 application is usually applied twice on both parents in the afternoon. The first application is made at 60-90 gm with 60-80 ppm per ha at 10% heading stage. The second application is made at 90-150 gm with 60-80 ppm per ha at 30% heading stage. However, in many countries outside China, the high cost of GA_3 application limits seed growers to use only 45-50 g ha^{-1}

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(Virmani *et al*., 2002). In the Philippines, three Mestizo hybrid combinations were commercially released from 1998 to 2001 using the CMS system. There were PSBRc72H (Mestizo 1, IR58025A x IR34686R), NSICRc114H (Mestizo 2, IR6888A x IR62161), NSCRc116H (Mestizo 3, IR688897 x IR60819R) (Redoña, 2002). For Mestizo 1, initial studies on GA_3 application conducted at Philippine Rice Research Institute (PhilRice) showed that using 17 gm GA_3 ha^{-1} applied in two splits did not make the panicle fully emerged from the leaf sheath resulting in a very low outcrossing rat and seed yield and which is not profitable for hybrid rice seed production (Suralta *et al*., 1999). Shen *et al*. (2000) reported that for IR58025A, spraying GA_3 at 180 gm/ha caused a panicle exsertion of about 2 t/ha. Pannuswamy *et al*.(1998) found an increasing proportion in seed set and yield by applying GA_3 significantly increased panicle exsertion, seed set and yield of CMS line IR58025A by a maximum of 80%, 20% and 77%, respectively at 150 g ha^{-1} dosage. Increase in seed yield was highly influenced by the increase in percentage of seed set presumably as a result of higher panicle exsertion, higher DPF (distance between panicle tip and flagleaf tip), wider flag leaf angle, higher degree of spikelet openings, and stigma exsertions (Suralta and Robles, 2004).

So far, there were very few researches concerning on GA_3 application for Mestizo 1 seed production, especially under wet season; for Mestizo 2 and 3, there is no research yet and it is a blank field which inhibits in enhancing the seed production yield and to expand to the new hybrid varieties. It is necessary, therefore, to develop the hybrid rice seed production using this study of concern.

Materials and Methods

Three experiments were conducted at Sto. Domingo, Nueva Ecija, Philippines during wet season 2005. Five dosages of GA_3 i.e. 0, 75, 150, 225, 300 g ha^{-1} applied and repeated three times in 48.4 m^2 plot area using Randomized Complete Block Design (RCBD). The parental lines were IR58025A and IR34686R for Mestizo 1, IR68888A and IR62161R for Mestizo 2 and IR68897 and IR60819R for Mestizo 3. The GA_3 powder used in the experiments was Chinese commercial product with 80% concentration.

According to the different growth durations of female parent (A line) and make parent (R line) and expecting heading stage, the different seeding date were designed to ensure flowering synchronization of both parents. Two splits sowing of R line were done with 6 days interval. The sowing interval between R_1 and A line was 20, 3, 9 days for Mestizo 1, 2, 3, respectively.

The age of seedling for transplanting was 23, 17, 20 days for R_1, R_2, A line, respectively. The R line seedlings were transplanted at 30 x 15 cm space and A line was 15 x 15 cm and the space was 20 cm between A and R line. The row ratio of 2:10 was used in the experiments. Each experiment was independently laid out in the field. The experimental area was isolated using more than 2 m height of polyethylene film as barrier during the flowering stage to ensure the F_1 seed purity.

Three (3) split applications of GA_3 using knapsack sprayer for every plot were done. First time spraying, when A line was at 5-10% heading stage, 20% dosage of the designed total amount of GA_3 was sprayed; Second time spraying, two days after first time spraying, A line heading about 30-40%, 50% dosage of total amount of GA_3 was sprayed; third time spraying, the day after second time spraying, A line heading about 60%, 30% dosage of total amount of GA_3 was sprayed. Two liters of GA_3 solution was applied for each plot in every time spraying.

Good field cultural management and practices were necessary. The fertilizer application of nitrogen, phosphorous and potassium was 130, 50, 80 kg ha^{-1}, respectively. 50% of total amount fertilizer was applied as basic fertilizer during the land preparation; the remaining amount of 40% fertilizer was applied for both parental plants 6 days after transplanting and 10% was individually applied to R line only. Roguing was done professionally in the whole growth duration to ensure the purity of hybrid seed and supplementary pollination was done three times each day from 9:30-12 am in seven (7) days after GA_3 application. The insects and diseases in the field were monitored for controlling, appropriate insecticide and fungicide was applied.

The samples from five hills each plot were selected randomly at maturity for the measurements which were related with the effects of GA_3 application in dosage. The data gathered was statistically analyzed using the analysis of variance (ANOVA) in RCBC to test the significance of the treatments that produced significant results were compared using the 5 percent level of significance of Duncan's Multiple Range Test (DMRT).

The experiments were conducted during wet season, too much rain and high relative humidity were the limiting factors to the hybrid rice seed production, which influenced the effect of GA_3 application and caused in lose of seed yield.

Results and Discussion

Plant height (cm)

The plant height of both parents were increased as the dosages of GA_3 application were increased. The higher the GA_3 dosage, the taller the differences among the treatments (Table 1, 2, 3). In general, the original plant height of R line is higher than A line and the sensitivity of R line to GA_3 is stronger than A line also. The result showed that the plant height of R line of Mestizo 1 treated with 300 g ha^{-1} was over 30 cm higher than A line which caused the panicles of R line to be easily broken by the wind and supplementary pollination rope which was observed in the experimental field. Moreover, during the flowering stage, the wind would easily dry the R line spikelets to bring on bloom 30-60 minutes ahead the female line which was observed also because the panicles of R line emerged out of the field plants through the wind alone due to high dosage of GA_3 application. Tian and Zhou (1991) stated that in the hybrid rice seed production, it is better if the plant height is not very tall; the standard depends on if the spikelet exsert from flag leaf sheath and keeps a difference of about 20 cm in plant height of female line and male line. The results about seed yield proved this opinion; the highest seed yield was not obtained by the plot treated with highest GA_3 dosage. In the hybrid rice seed production, if it is necessary to require high GA_3 dosage for some female lines or the original plant height of R line was much higher than A line plants, such as Mestizo 1, GA_3 application on R line than A line.

Length of enclosed panicle segment in the leaf sheath (LEPS)

The LEPS of A line in the three experiments is significantly influenced by GA_3 application. The effect of GA_3 dosage indicates that as GA_3 dosage increased there was a corresponding decrease in the LEPS (Table 1, 2, 3). However, in the Mestizo 1 seed production, result showed that all the dosages of GA_3 application still cannot induce complete panicle exsertion, even with the highest dosage (300 g ha^{-1}) of GA_3 application still resulted in (1.79 cm) panicle segment in the leaf sheath. It required higher dosage to fully emerge the panicle segment. Whereas, in the Mestizo 2 experiment, the LEPS had a negative (-1.33 cm) when applied with 300 g ha^{-1} which means that the panicle fully emerged from the leaf sheath and the neck node of the panicle had already emerged from the opening of the leaf sheath. The condition is the same in the Mestizo 3 experiment, the LEPS was negative with -0.36 cm and -3.22 cm when applied 225 g ha^{-1} and 300 g ha^{-1} GA_3, respectively. This indicates that the effect of GA_3 application in term of LEPS was more effective to the A line of Mestizo 2 and Mestizo 3 than to Mestizo 1.

If LEPS obtained negative, the panicle that emerged is too much from the leaf sheath, it would easily be broken by wind or rain and would result to less seed yield, especially during the wet season (Yuan, 1985). In general, there is a distance of about 2-3 cm from panicle neck node to the lowest spikelet of panicle; the best effect to LEPS was not 100% panicle exsertion but to just completely emerge all the spikelets and the panicle segment enclosed with about 2-3 cm by leaf sheath, which avoided panicle disjunction. Based on this, the best GA_3 dosage to panicle exsertion was 225-300 g ha^{-1}, 150-225 g ha^{-1} and 150 g ha^{-1} for M_1, M2 and M_3, respectively. GA_3 promote panicle exsertion through rice cell elongation and division (Bidwell, 1974). The effect of GA_3 on panicle peduncle is to change the intracellular concentration of solutes, which causes the osmotic influx of water into the cell resulting in increased turgor. Plant cells may expand as a response to an increase in turgor pressure against the cell wall (Michael and Sunday, 2005).

Table 1. Agronomic characteristic, yield and yield components of Mestizo 1 seed production as affected by different dosages of GA_3 application (WS 2005).

Treatment	PH (cm)		LEPS	PES	NTSP	WSG	PSES	SLR	PSS	SY
(GA_3 dosage)	A line	R line	(cm)	(%)	(gm)	(gm)	(%)	(day)	(%)	(kg/ha)
T_1 (0, control)	90.45^{c}	110.56^{d}	10.18^{a}	28.12^{a}	146.4	18.67	24.60^{d}	5	6.26^{c}	224.85^{c}
T_2(75 gm/ha)	99.84^{b}	120.88^{c}	7.49^{b}	18.81^{b}	144.7	18.53	32.85^{c}	7	12.17^{b}	429.75^{b}
T_3(150 gm/ha)	103.59^{b}	129.44^{b}	5.98^{c}	12.98^{c}	142.7	18.60	43.94^{c}	7	13.41^{b}	530.30^{b}
T_4(225 gm/ha)	115.49^{a}	140.50^{a}	3.75^{d}	3.98^{d}	150.6	18.80	53.17^{a}	7	28.66^{a}	986.23^{a}
T_5(300 gm/ha)	118.85^{a}	148.78^{a}	1.79^{e}	1.65^{d}	145.1	18.53	45.66^{b}	7	29.20^{a}	965.56^{a}

Table 2. Agronomic characteristic, yield and yield components of Mestizo 2 seed production as affected by different dosages of GA_3 application (WS 2005).

Treatment	PH (cm)		LEPS	PES	NTSP	WSG	PSES	SLR	PSS	SY
(GA_3 dosage)	A line	R line	(cm)	(%)	(gm)	(gm)	(%)	(day)	(%)	(kg/ha)
T_1 (0, control)	81.55^{c}	84.75^{e}	8.63^{a}	21.14^{a}	127.3	20.87	17.48^{c}	5	7.20^{c}	330.58^{c}
T_2(75 gm/ha)	105.94^{b}	112.80^{d}	6.03^{b}	13.55^{b}	119.8	20.93	33.14^{b}	7	13.60^{b}	623.28^{b}
T_3(150 gm/ha)	112.65^{b}	121.56^{c}	3.83^{bc}	9.42^{b}	124.1	20.87	46.88^{a}	7	23.56^{a}	891.87^{a}
T_4(225 gm/ha)	117.71^{ab}	135.92^{b}	2.06^{c}	1.64^{c}	129.3	21.27	48.48^{a}	7	24.61^{a}	902.89^{a}
T_5(300 gm/ha)	121.27^{a}	143.40^{a}	-1.33^{d}	0.73^{c}	118.2	20.60	38.98^{a}	7	22.22^{a}	798.91^{a}

Percentage of enclosed spikelet in the leaf sheath (PES)

The PES of A line affected by GA_3 application showed similar variation with LEPS, the PES was decreased as the GA_3 dosage increased. There were highly significantly differences among the treatments (Table 1, 2, 3). Yan (1999) stated that PES is decreased by GA_3 application as low as possible in the seed production which ensures maximum spikelets exposure from leaf sheath for pollination. Result showed the lowest PES obtained from plants treated with 300 g ha^{-1} had high significant difference with 150 g ha^{-1} but no significant difference with 225 g ha^{-1} for Mestizo 1 (Table 1) and Mestizo 2 (Table 2) which expresses that application of 225 g ha^{-1} dosage was more than 300 g ha^{-1}. Also, the similar condition in Mestizo 3 (Table 3) indicates that application of 150 g ha^{-1} dosage was more effective and economical in reducing PES.

Table 3. Agronomic characteristic, yield and yield components of Mestizo 3 seed production as affected by different dosages of GA_3 application (WS 2005).

Treatment (GA_3 dosage)	PH (cm)		LEPS (cm)	PES (%)	NTSP (gm)	WSG (gm)	PSES (%)	SLR (day)	PSS (%)	SY (kg/ha)
	A line	R line								
T_1 (0, control)	90.29^{d}	105.50^{e}	7.19^{a}	18.00^{a}	130.7	22.67	29.42^{d}	6	8.90^{c}	425.62^{c}
T_2(75 gm/ha)	102.88^{c}	116.00^{d}	5.85^{a}	10.94^{b}	133.9	23.07	35.65^{c}	8	15.15^{b}	694.22^{b}
T_3(150 gm/ha)	114.57^{b}	130.44^{c}	2.83^{b}	2.98^{c}	134.9	22.80	48.66^{a}	8	27.46^{a}	1029.6^{a}
T_4(225 gm/ha)	119.88^{ab}	138.72^{b}	-0.36^{c}	1.67^{cd}	137.0	22.87	41.30^{b}	8	28.63^{a}	1020.7^{a}
T_5(300 gm/ha)	122.51^{a}	147.32^{a}	-3.32^{d}	0^{d}	136.3	22.73	39.40^{bc}	8	24.95^{a}	938.04^{a}

Percentage of spikelet with exserted stigma (PSES) and stigma longevity and receptivity (SLR)

The result showed GA_3 application highly affected the PSES and SLR. Chen (2000) stated that the PSES and SLR were very important factor which influenced the seed set rate, high PSES and SLR would increase the stigmas' chance in accepting pollens from male parents which would increase the female parental seed yield. Hence, it was necessary to improve the PSES and SLR as high as possible in the seed production. For the A line of Mestizo 1 (Table 1) and Mestizo 3 (Table 3), the highest PSES with 53.17%, 48.66% was obtained by the plants applied with 225 g and 150 g ha^{-1} of GA_3 respectively and there were significant differences with other treatments. This inducates that 225 g ,150 g ha^{-1} was the optimal GA_3 dosage to increase the PSES for Mestizo 1 and 3, respectively. For the A line of Mestizo 2 (Table 2), plants applied with 225 g ha^{-1} of GA_3 obtained the highest PSES with 48.48% but had no significant difference with plants treated with 150 and 300 g ha^{-1}. This indicates that 150 g ha^{-1} of GA_3 dosage was more effective and economical to increase the PSES for Mestizo 2.

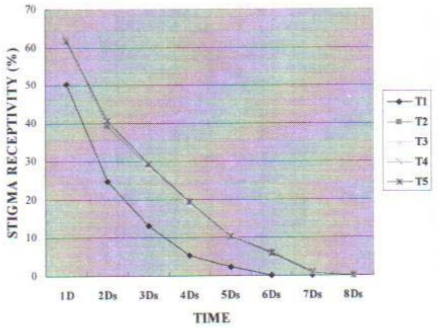
GA_3 application extened stigma longevity span for 2 days and greatly enhanced the stigma receptivity in each variety (Figure 1, 2, 3). But there were similar effects and no significant difference among treatments with different GA_3 dosages. The low GA_3 dosage with 75 g ha^{-1} had the same SLR with 300 g ha^{-1}. It was known that improving SLR only needed a small amount was not required. The PSES and SLR depend on the vigor of stigma during the flowering stage. An effect of exogenous GA_3 on rice genotypes was the stimulation and inhibition of the activity of different enzymes. Exogenous GA_3 caused a greater reduction in contents of protein and total organic N, P and Fe in organs of dwarf rice genotypes; whereas it markedly enhanced the level of total carbohydrate and starch in the rice plants (Singh and Ram, 1997) which highly enhanced the vigor of stigma to keep longer longevity and higher receptivity to the male pollens.

Number of total spikelet per panicle (NTSP) and weight of 1000 seed grains (WSG)

Result showed that the NTSP and WSG of A line were not influenced by GA_3 application. The data of NTSP and WSG applied with any GA_3 dosage were similar in each Mestizo varieties. There was no significant difference among all the treatments (Table 1, 2, 3). This agrees with Chen (1995), who stated that the NTSP and WSG were the stable inherited characters in rice variety and were influenced by parents' genes.

Percentage of seed set (PSS)

The PSS of A line was very low without GA_3 application resulting in very low seed yield, as in A line of Mestizo 1. The lowest PSS was obtained from the untreated control plants with 6.26% only which was obviously lower than the mean of other treatments applied with GA_3 which is 20.86%. GA_3 application is effective in increasing the PSS. In hybrid rice seed production, it is better to increase the PSS as high as possible. Result showed (Table 1, 2, 3) that the optimal GA_3 dosage obtained the highest PSS and most economical were 225, 150, 150 g ha^{-1} for Mestizo 1, 2, 3, respectively. But the highest PSS in these experiments was less than 30%. It might be caused by too much rain and strong wind during the flowering stage. However, Tian and Zhou (1991) reported that the PSS was not directly affected by GA_3 application. GA_3 application greatly influenced the plant height, panicle and spikelet exsertion, stigma exsertion, stigma longevity and receptivity, which improved the outcrossing condition and directly affected the PSS.



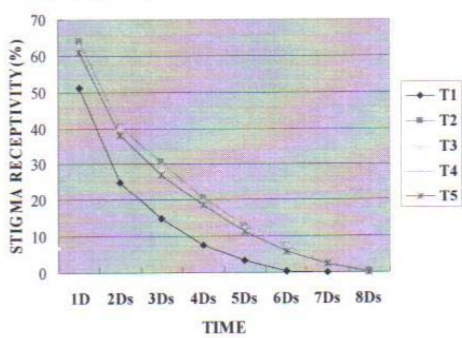
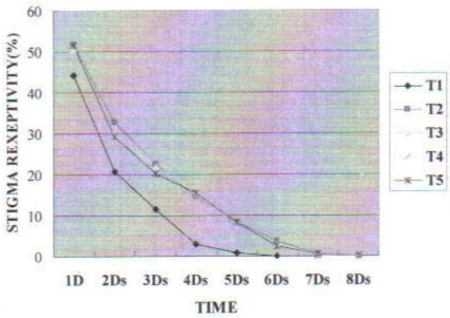


Fig 1. Stigma longevity and receptivity of the A line of the Mestizo 1 seed production as affected by the different dosages of GA_3 application.

Fig 2. Stigma longevity and receptivity of the A line of the Mestizo 2 seed production as affected by the different dosages of GA_3 application.



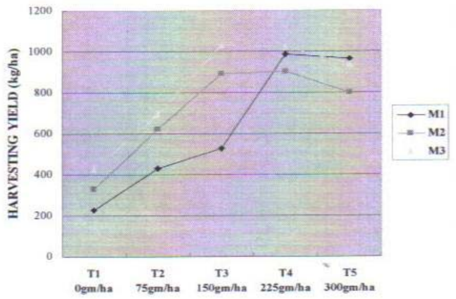


Fig 3. Stigma longevity and receptivity of the A line of the Mestizo 3 seed production as affected by the different dosages of GA_3 application.

Fig 4. Seed yield of the A line of the Mestizo 1, 2, 3 as effected by the different dosages of GA_3 application.

Seed yield (SY)

Seed yield was composed of number of hill/ha x number of productive panicle/hill x number of spikelet/panicle x seed set rate x weight of 1000 grains. Seed set rate was only one component affected by GA_3 application in five components. In hybrid rice seed production, the highest/lowest PSS corresponds to highest/lowest seed yield (Tian and Zhou, 1991). The experimental results had met this opinion. For A line seed yield of Mestizo 1 (Table 1), the highest seed yield (986.23 kg ha^{-1}) was recorded from treatment applied with 225 g ha^{-1}, which was 4 times higher than the untreated control (224.85 kg ha^{-1}). The extent of increased in seed yield was especially high and there was highly significant difference. For A line seed yield of Mestizo 2 (Table 2), the highest seed yield with 902.89 kg ha^{-1}, was obtained from plants applied with 225 g ha^{-1}, but the effects of GA_3 application with 150 g ha^{-1} and 225 g ha^{-1} were similar in increasing the seed yield. Thus, the GA_3 dosage of 150 g ha^{-1} was the most effective and economical to reach high seed yield. For A line seed yield of Metizo 3 (Table 3), results showed that the highest seed yield with 1029.6 kg ha^{-1} was obtained by the plants treasted with 150 g ha^{-1} of GA_3 and found the most effective and economical in increasing seed yield.

Conclusion

GA_3 application was very effective in increasing seed set rate and seed yield through elongation of plant height, promoting panicle and spikelet exsertion, enhancing stigma exsertion and longevity and receptivity. During the 2005 wet season in the Philippines, 225, 150, 150 g ha^{-1} of GA_3 dosage were found the most effective and economical thus recommended for Mestizo 1, 2, 3 seed production, respectively.

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