Effect of GA_3 Concentration on Hybrid Rice Seed Production in Indonesia

Pepi Nur Susilawati
Banten Assesment Institute for Agricultural Technology
Jl. Ciptayasa KM 1
Ciruas Serang, Banten
Indonesia.

Memen Surahman

Bambang S. Purwoko

Tatiek K. Suharsi

Department of Agronomy and Horticulture
Faculty of Agronomy, Bogor Agriculture Institute
Jl. Meranti, Kampus IPB Darmaga 16680
Indonesia

Satoto
Rice Research Center
Jl. Raya Sukamandi Km 5
Subang-Jawa Barat
Indonesia

Abstract

In Indonesia, hybrid rice was developed with three line system however F1 seed production levels are still low. GA_3 has been proved increase the seed set through increased of panicle exertion and improved outcrossing. The purpose of this study is to identify the appropriate doses of GA_3 in increasing F1seed production. The material comprising both the parental lines (cytoplasmic male sterlity and restorer) of hybrid HIPA 8 (A1 and BP51-1), HIPA 6 (A2 and B8094), HIPA jatim 3 (A6 and PK88) and HIPA 14 SBU (A7 and BH33d-Mr-57-1-2-2). GA_3 powder used in the experiment is 90% concentration in four level consentration i.e. 0, 100, 200, 300 ppm. The results showed that GA_3 positively increase plant height, stigma exertion, panicle exertion, duration of floret opening, angle of floret opening and panicle length. Increased seed production is 0.2 to 1 t/ha compared to the control (0 ppm GA3). GA_3 concentration of 200 ppm gave the best results as indicated by the highest productivity of seed set and yield in all CMS lines were tested.

Keywords : GA_3, CMS, hybrid rice.

Introduction

Rice is an important crop for food security around the world. In Indonesia, rice demands is very high because most of the population consumes rice. Efforts to meet the rice needs can be done in two ways: expanding the rice planting area and increased production, or both. But in the future, expansion will be more difficult and expensive, substantial improvement can be done through the adoption of hybrid rice (Nguyen 2010).

The success in increasing rice production through hybrid rice has been proven in China. The increase in production is 15-20% higher than the best commercial inbred rice, with a planting area reached more than 50% of the total area (You *et al*. 2006). Hybrid rice has contributed significantly to food safety in China in the last 25 years. Following the success in China, Indonesia also developing hybrid rice technology since 1983 and began to develop in 2001. In 2008, Indonesia has Rice Research Center has been released 35 varieties of hybrid rice. The release of hybrid rice varieties aims to accelerate rice self sufficiency as proclaimed by the government of Indonesia (Satoto *et al*. 2010).

Rice is autogami plant so that the level of cross breeding is naturally low (Sheeba et al 2006), therefore the production of hybrid rice developed by male sterility system. The following genetic system are known for developing rice hybrids are two system production. First, cytoplasmic-genetic male sterility (CMS) for developing three-line system hybrid rice. Second, photo and/or thermo-sensitive genetic male sterility (PGMS or TGMS) for developing two-line sistem hybrid rice (Yuan *et al* 2003).

In Indonesia, hybrid rice developed by the three line systems. The weakness of this system is F1seed production level is low. The low of seed production due to lack of panicle exertion and low outcrossing.

Application of GA_3 is an effective plant growth hormone wich stimulates the cells elongation. GA_3 is an key to win high seed yield in hybrid rice seed production It can make increase panicle exertion from the flag leaf, increase the rate of stigma exertion, adjust plant hight, increase the duration of floret opening and make the later taller and productive (Virmani and Sharma 1993; Yuan *et al*. 2003; Viraktamath dan Ilyas 2005, Gavino *et al* 2008).

Effect of GA_3 on the growth and development of plants is determined by the varieties and environment. In a new growing environment such as in the Province of Banten, it is necessary to identify the appropriate concentration of GA_3 for some hybrid rice varieties. Therefore it is necessary to conduct a research to study the effect of GA_3 concentration on some hybrid rice varieties. This research was expected provide some information concerning appropriate GA_3 concentration levels to support the process of pollination between male sterile lines with restorer in Province of Banten, Indonesia.

Materials And Methods

The experiment were conducted at KP Singamerta, BPTP Banten Province in Indonesia, during December 2012 until March 2013. The material comprising both the parental lines of hybrid Hipa 8 (A1 and BP51-1), Hipa 6 (A2 and B8094), Hipa jatim 3 (A6 and PK88) dan Hipa 14 SBU (A7 and BH33d-Mr-57-1-2-2) . The GA3 powder used in the experiment with 90% concentration in four level concentration i.e. 0, 100, 200, 300 ppm.

The age of seedling for transplanting was 21 days for CMS (A) and restorer (R) lines. Single rows of A line was sown followed by three rows of R line at three-days interval, A line was also sown at six days interval (Hipa 5, Hipa 6 and Jatim 3) and nine days interval (Hipa 14 SBU) from last sowing of R line. Subsequently, the seedlings were mixed in equal proportion to the three dates of sowing before transplanting. Restorer line was transplanted in paired rows with three seedling per hill keeping 30x15 cm spacing and A line was 15x15 cm and space was 20 cm between A and R line, in a plot of 4x5 m^2. The row ratio of 2:8 was used in the experiments. Each experiment was independently laid out in the field. The experiment area was isolated using more than 3 m height of polyethylene plastic as barrier during the flowering stage ensure the F1 seed purity.

GA3 were sprayed in two spraying using knapsack sprayer for every plot were done. First time spraying, when A line was at 5-10% heading stage. Second time spraying three days after first time spraying. One, which remained untreated, was used as the control. The data were recorded on 10 randomly selected plants from each replications for nine quantitative characters. The characters studied were plant height (cm), effective tillers per plant, panicle length (cm), panicle exertions of male sterile lines (%), stigma exertions of male sterile line (%), seed set (%), angle of floret opening (^{o}), duration of floret opening (minutes) and seed yield (kg/ha). The general reference for data collection was standard evaluation system for rice (SES) (IRRI, 2002). The data gathered was statistically analyzed using the analysis of variance (ANOVA) in Split Plot Design (Main plot was parental lines and sub plot was GA3 dosages) to the test the significance for each character. If the treatment that produced significant results were compared using the 5 percent level of significance of Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1993).

Results And Discussion

Analysis of variance showed that GA_3 concentration significantly affect all observed parameters except number of tillering. The interaction between varieties and GA_3 concentration shown in panicle length, panicle exertion, stigma exertion, seed set and seed yield (Virmani, 1998, Biradarpatil and Shekhargouda 2006, Tiwari et al, 2011). GA_3 also affect the panicle exertion, flowering character such as stigma exertion and duration of floret opening (Gavino, 2008; Rumanti, 2012)

Plant height greatly influenced by GA_3 spraying, at a concentration of 100 ppm the increase rate of plant height ranged from 11.56 to 13.56 cm.

GA_3 concentration of 200 ppm is able to increase the plant height of 14.60 to 25.10 cm, whereas at a concentration of 300 ppm increase in plant height is 24.28 to 29.32 cm (Table 1). The increase in plant height due to increased activity of cells division, enlargement and elongation. Gibberellins are plant hormones thar regulate various processes of plant growth and development, which is particularly important in stem elongation (Hedden and Phillips 2000; Sakamoto et al., 2004; Sun 2004; Tiwari et al. 2011).

Table 1. Agronomic traits of four male sterile lines in several different applications of GA_3 concentration (Pooled data of two seasons).

Lines	Concentration of GA3 (ppm) 0	100	200	300	Average
	----------Plant height (cm)----------				
A1	84.34	97.00	109.44	113.67	101.11
A2	95.11	108.67	118.11	119.89	110.45
A6	96.22	109.78	110.82	122.87	109.92
A7	100.22	111.78	122.44	128.32	115.69
Average	93.97^{b}	106.81^{ab}	115.21^{a}	121.11^{a}	
	----------Effective tillers per plant----------				
A1	16.55	18.67	18.55	17.22	17.75^{b}
A2	24.78	24.56	23.11	23.44	23.97^{a}
A6	24.89	24.94	24.67	23.89	24.60^{a}
A7	21.78	19.72	18.73	22.23	20.62^{ab}
Average	22.00	21.97	21.27	21.70	
	----------Panicle length (cm)----------				
A1	25.74 ^{d}	28.19 ^{b}	28.66 ^{b}	30.58^{a}	28.29
A2	21.47 ^{g}	24.39 ^{e}	25.12d^{e}	25.79^{d}	26.69
A6	22.99 ^{f}	25.74 ^{d}	26.58 ^{cd}	26.93^{c}	25.56
A7	24.39 ^{e}	27.23 ^{c}	27.55 ^{bc}	28.35 ^{b}	26.89
Average	23.65	26.39	29.48	27.92	
	----------Panicle exertions (%)----------				
A1	75.55^{f}	83.33^{d}	87.27^{c}	88.69^{bc}	83.71
A2	74.79^{f}	83.31^{d}	88.00^{bc}	90.26^{ab}	84.09
A6	77.42^{ef}	86.23^{c}	89.37^{b}	92.43^{a}	86.36
A7	79.82^{e}	82.84^{d}	86.42^{c}	88.44^{bc}	84.38
Average	76.90	83.93	87.77	89.96	
	----------Flowering age 50% (HSS)----------				
A1	85.00	85.00	85.00	85.00	85.00
A2	76.00	76.00	76.00	76.00	76.00
A6	74.00	74.00	74.00	74.00	74.00
A7	78.00	78.00	78.00	78.00	78.00
Average	78.25	78.25	78.25	78.25	

Values with different letter(s) within a column differ significantly at 5% level probability (LSD)

Panicle length and panicle exertion influenced by the interaction between varieties with GA_3 concentration. GA_3 spraying at 100, 200, 300 ppm significantly increased the panicle length compared to controls on all varieties (Table 1). Panicle elongation due to GA_3 spraying ranged from 2.45 to 4.84 cm. GA_3 increase panicle length due to cell division and elongation (Yuan *et al*. 2003; Tiwari *et al*. 2011).

Panicle exertion is measured from panicle length coming out flag leaf sheath compared with the total length of panicle. GA_3 application significantly show positive influence on the increase of panicle exertion for all hybrid varieties tested. GA_3 at 200 and 300 ppm shows the highest effect (86.42 to 92.43%) compared to controls (74.79 to 79.82%) and 100 ppm (82.84 to 86.23%). Study of Yin et al (2007) has showed the effect of GA_3 on panicle base elongation of male sterile lines and panicle can be filled out from the flag leaf sheath so that opportunities of seed formation become larger.

In this study, panicles exertion not reach 100%, but at GA_3 concentrations of 200 and 300 ppm almost all spikelet emerge. Research on male sterile lines namely Mestizo1, Mestizo Mestizo 2 and 3 show the best effect of GA_3 on panicle exertion if the exertion percentage does not reach 100%, but spikelet can still appear.

Percentage of panicles exertion 100% can result in brittle panicle by wind and rain so it will lower the yield, especially in the rainy season (Gavino *et al*. 2008).

Increased panicle exertion positively improve seed set. Panicles exertion will increase the chances of seed set because part of the pancile covered with flag leaf will be open and allow the cross breeding. Jagadeeswari *et al*. (1998) stated that the application of GA_3 on male sterile lines could increase panicle exertion by 20 to 30% thereby increasing the grain yield as much as 35 to 60%. In this study, increase in seed set due to GA_3 spraying ranged from 5.7 to 48.8% (Table 2).

Stigma exertion was observed by counting the number of flowers that have stigmas remain outside when the flowers have finished blooming (lemma and palea closed). Stigma exertion is positively influenced by an increase in the concentration of GA_3. Stigma exertion will affect the formation of seeds (seed set), it is because the stigmas that are outside when the flowers has been closed lead to be pollinated for several days.Increasing GA_3 concentrations will be followed by an increase of stigma exertion on all lines tested (Table 2). CMS A1 experiencing stigma exertion increased at concentrations of 100, 200 300 ppm namely 42.69, 60.56 and 67.65% respectively. CMS A2 experiencing stigma exertion increased by 24.54, 34.29 and 55.87%. Increased stigma exertion of 19.53, 22.57, 29.12% found at CMS A6 and by 38.21, 40.46, 53.47% found at at CMS A7. Based on these results it appears that CMS A1 and A7 most responsive to GA_3 spraying.

The duration and angle of floret opening significantly affected by GA_3 spraying compared with controls. The duration and angle of floret opening affect the successful formation of seeds (seed set), this is because the chances of cross pollen will be longer and larger. Previous studies showed that high degree of cross pollination in hybrid rice affected by the long duration of floret opening, large stigma surface and angle of floret opening (Singh and Shirisha 2003; Biradarpatil and Shekargouda 2006; Gavino *et al*. 2008). allow for a longer pollination.

Table 2. Stigma exertion, duration of floret opening, angle of floret opening, seed set, and seed yield of four male sterile lines in several different applications of GA_3 concentration (Pooled data of two seasons).

Lines	Concentration of GA3 (ppm) 0	100	200	300	Average
	----------Stigma exertions (%)----------				
A1	34.05^{e}	48.58^{cd}	54.67^{abc}	57.08^{ab}	48.59
A2	33.85^{e}	42.10^{d}	45.49^{d}	52.76^{b}	43.56
A6	47.52^{cd}	56.80^{ab}	58.25^{ab}	61.36^{a}	55.98
A7	41.71^{d}	57.65^{ab}	58.59^{ab}	59.84^{ab}	54.45
Average	39.28	51.30	54.25	57.76	
	----------Duration of floret opening (minute)----------				
A1	49.41	91.07	97.70	102.05	85.06^{ab}
A2	63.41	63.81	65.00	67.89	65.03^{b}
A6	65.67	82.33	97.22	120.07	91.32^{a}
A7	39.06	62.61	64.22	82.33	62.06^{b}
Average	54.39^{b}	74.96^{ab}	81.04^{a}	93.20^{a}	
	----------Angle of floret opening (^{o})----------				
A1	24.5	27.5	29.33	29.17	27.63
A2	24.44	28.56	30.83	32.92	29.19
A6	22.67	26.75	30.33	26.69	26.61
A7	25.58	30.75	32.5 0	32.36	30.30
Average	24.30^{b}	28.39^{ab}	30.75^{a}	30.29^{a}	
	----------Seed set (%)----------				
A1	17.31 ^{h}	18.3 ^{h}	23.69 ^{de}	21.93 ^{ef}	20,31
A2	13.31 ^{k}	15.38 ^{i}	17.56 ^{h}	18.85 ^{gh}	16,28
A6	16.26 ^{h}	20.93 ^{fg}	24.12 ^{d}	23.22 ^{de}	21,13
A7	24.45 ^{d}	26.72 ^{c}	34.29 ^{a}	32.45 ^{b}	29,48
Average	17,83	20,33	24,92	24,11	
	----------Seed yield (kg/ha)----------				
A1	830de	1240^{cd}	1550^{b}	1600^{b}	1305.00
A2	332,5^{e}	540de	950^{cd}	900^{cd}	680.63
A6	670de	1100^{cd}	1450^{bc}	1400^{bc}	1155.00
A7	720de	980^{cd}	2120^{a}	1800^{ab}	1405.00
Average	638.13	965.00	1517.50	1425.00	

Values with different letter(s) within a column differ significantly at 5% level probability (LSD)

Seed yield (Table 2) showed that all CMS tested resulted in higher productivity after GA_3 application compared with controls. GA_3 concentration of 200 ppm tend to give higher productivity results than 300 ppm. This occurs because panicle stalk broken due to rain and wind resulting in yield loss. GA_3 applications in high concentrations potentially increase panicle broken because of increase in plant height and panicle, especially during the rainy season (Yuan 1985; Prasad *et al*. 1988; Gavino *et al.* 2008). Therefore, GA3 application must be adapted with responsiveness of male sterile lines, agro-ecological conditions and seasons.

Conclusion

Treatment using three differentt GA_3 concentration positively increase plant height, stigma exertion, panicle exertion, duration of floret opening, angle of floret opening and panicle length. Improved some flowering and growth trait able to increase seed production by 0.2 to 1 t/ha.

GA_3 concentration of 200 ppm gave the best results compared to the control and other GA_3 concentration as indicated by the highest seed set and productivity in all CMS tested. Concentration of 300 ppm GA_3 resulted in increased cell elongation that make rice susceptible to fall and panicle easily broken in a large rain and wind conditions. 200 ppm of GA_3 is recommended to be applied for hybrid rice seed production in Banten province Indonesian.

Acknowledgements

The authors thank to the Agency for Agricultural Research and Development, Center for Agricultural Technology Assessment and Development, Institute for Agricultural Technology, Province of Banten, Ministry of Agriculture which has provided funding for the study and research.

References

Biradarpatil NK, Shekhargouda. 2006. Cost effective techniques for enhancing seed yield and quality of hybrid rice. Karnataka J.Agric.Sci. **19**(2):291-297.

Gavino RB, Pi Y, Abon Jr CC. 2008. Application of geberellic acid (GA3) in dosages for three hybrid rice seed production in the Philippines. *Journal of Agricultural Technology*. **4**(1): 183-192.

Hedden P, Phillips AL. 2000. Gibberellin metabolism: New insight revealed by the genes. *Trends Plant Sci*. **5**:532-530.

[IRRI] International Rice Research Institute. 2002. Standard evaluation system of Rice (SES). IRRI, Philippines.

Jagadeeswari P, Kumar SS, Ganesh M, Anuradha G. 1998. "Effect of foliar application of giberelic acid on seed yield and quality in hybrid rice". Oryza, **35**: 26-30.

Jagadeeswari P, Sharma SP, Dadlani M. 2004." Effect of different chemicals on traits favouring outcrossing and optimization of GA3 for seed production of cytoplasmic male sterile line in hybrids rice". Seed Sci. and Tecnol. **32**: 473-483.

Nguyen NV. 2010. Ensuring food security in the 21st century with hybrid rice: Issues and Challenges. In: Accelerating Hybrid Rice Development. Xie F, Hardy B. (eds). International Rice Research Institute. Los Banos. Philippines. Pp:9-24.

Prasad MN, Virmani SS, Gamutan AD. 1988. "Substituting urea and borac acid for gibberelic acid in hybrid rice seed production". International Rice Research Notes. **13**(6): 9.

Rumanti AR. 2012. Pengembangan galur mandul jantan dengan tiga sumber sitoplasma untuk perakitan padi hibrida. Disertasi. Institut Pertanian Bogor.

Sakamoto T, Miura K, Tatsumi T, Ueguchitanaka M, Ishiyama K. 2004. An overview of gibberellin metabolism enzyme and their related mutants in rice. *Plant Physiol*. **134**:1642-1653.

Satoto, Sutaryo B, Suprihatno B. 2010. Prospek pengembangan varietas padi hibrida . Dalam : Darajat, *et al*., (Eds). Padi : Inovasi teknologi produksi. Buku 2. Balai Besar Penelitian Tanaman Padi. Sukamandi. Subang. hlm. 29-65.

Sheeba A, Vivekanandan P, Ibrahim SM. 2006. Genetic variability for floral traits influencing outcrossing in the cms lines rice. Indian J. Agric. Res. **40**(4):272-276

Steel RG, Torrie JH. 1993. Prinsip dan prosedur statistika. PT. Gramedia Pustaka Utama, Jakarta. 748 p.

Sun T. 2004. Gibberellin Signal Transduction in stem elongation and leaf growth. In: Plant Hormones, Biosynthesis, Signal transduction, Action, Davies P.J. (eds). Kluwer Academic Publ. Dordrecth. Netherlands. Pp:304-320.

Tiwari DK, Pandey P, Giri SP, Dwivedi JL. 2011. Effect og GA3 and other plant growth regulators on hybrid rice seed production. Asian Journal of Plant Sciences **10**(1):1-7.

Viraktamath BC, Ilyas A. 2005. "Principal Scientist of Hybrid Rice". Training of Hybrid Rice Seed Production. Sukamandi - Indonesia 14 – 18st July 2005.

Virmani SS, Sharma HL. 1993. Manual for Hybrid Rice Seed Production. IRRI. Manila. Philippines. 72 p.

Yin C, Gan L, Denny Ng, Zhou X, Xia K. 2007. Decreases panicle-derived indole-3-acetic acid reduces gibberelin A1 level in the uppermost internode, causing panicle enclosure in male sterile rice Zhenshan 97A. Journal of Experimental Botany **58**(10):2441-2449.

You A, Lu X, Jin H, Ren X, Liu K, Yang G, Yang H, Zhu L, He G. 2006. Identification of quantitative traits loci across recombinant inbred lines and testcross populations for traits of agronomic importance in rice. *Genetiks* 172:1287-1300.

Yuan L, Wu X, Liao F, Ma G, Xu Q. 2003. Hybrid Rice Technology. *China Agricultural Press. Beijing*, China. 132p.