International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 1603-1607

Received: 08-05-2017
Accepted: 10-06-2017

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Assessment of appropriate dosage of gibberellic acid (GA_3) for better seed yield of a promising hybrid rice variety

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Abstract
The present investigation was carried out during *kharif* 2013-2014 at Norman E. Borlaug Crop Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. In present Investigation Parental lines of Pant Sankar Dhan-3 *i.e.* UPRI 95-17A (Female line), UPRI 93-287R (restorer line) were sprayed with four GA_3 treatments *i.e.* T_1 – GA_3 @ 60 gm/ha, T_2- GA_3 @ 90gm/ha, T_3- GA_3 @120gm/ha and T_4- Control. The objective of the study was to determine optimum dose of GA_3 application on seed parent for better seed yield. Experiment revealed that all the characters showed the increasing trend with increasing GA_3 dose in field, except number of spikelets per panicle and 1000 seed weight. Germination per cent and other vigour parameters of hybrid rice seed reduced significantly with the increased dose of GA_3 and storage period. Highest germination and vigour parameters were observed in T_4 (control) and lowest in T_3 (120g GA_3/ha) at the end of storage period. More yield can be obtained by using GA_3 dose 120g/ha (T_3). But, it will not be economically viable and high dose of GA_3 also adversaly affaected the seed germination and vigour during storage. While, GA_3 dose 60g/ha (T_3) also gave the significant yield increase over control and gradual loss of germination and vigour was also observed in T_3 (60g GA_3/ha) in comparison to other GA_3 treatments.

Keywords: Gibberelic acid (GA_3), cytoplasmic male sterile (CMS) lines, hybrid rice, Pant Sankar Dhan-3, Wild abortive (WA)

Introduction
Rice (*Oryza sativa* L.) is the staple food source about half of the world's population. As the world's population is continuously increasing, there will be need of further increase in rice production to meet out the additional food demand of growing population. Among the various possible approaches, hybrid rice cultivation is the most feasible and practical one as it has yield advantage of 20-30% over conventional high yielding varieties. Among various hybrid developing approaches, Cytoplasmic Genetic Male Sterility (CGMS) system, is being widely adopted because it is the most common, easy and effective method for developing new hybrids in the hybrid rice breeding programme. The weakness of the three line systems of hybrid rice development is low level of F_1 seed production.

The low rate of seed production is due to poor panicle exertion causing reduced out crossing and seed setting in the cytoplasmic male sterile (CMS) lines, since most of the the CMS lines based on the wild abortive (WA) cytoplasm have imperfect panicle exsertion, at least 25-30% spikelets remain enclosed in the flag leaf thus reducing the total number of spikelets available for out crossing (Swain and Rao 2009) [1]. Poor panicle exertion is caused by poor elongation of the last internode just below the panicle. Increased seed yield can be witnessed, if the panicle exertion is increased thereby facilitating more number of spikelets for seed setting. Shengqui (1988) [17]. Proposed application of GA_3 to obtain high seed yield in CMS line. Application of GA_3 can increase panicle exertion from the flag leaf, stigma exertion, increase the duration of floret opening, adjust plant height, and make the later tiller taller and productive (Virmani and Sharma 1993; Yuan *et al.* 2003; Gavino *et al.* 2008) [4, 3, 2].

To utilize the advantage of hybrid rice, it is necessary to develop a cost effective seed production system successfully. Presently, use of Gibberellic acid is almost the prerequisite for hybrid rice seed production which increases the cost of hybrid seeds. So, it is necessary to determine appropriate doses of GA_3 for its commercial feasibility during seed production. Keeping in view of these needs the present study was undertaken to determine the appropriate dose of GA_3 for female parent and residual effect of GA_3 on seed germination and vigour of

Hybrid seed.

Materials and Methods

The present investigation was carried out during *Rabi* 2014 at Norman E Borlaug Crop Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, District Udham Singh Nagar, Uttarakhand, India. Parental lines of Pant Sankar Dhan-3 *i.e* UPRI 95-17A (Female line), UPRI 93-287 R (restorer line) were taken for the experiment. The experiment was laid out in Randomized Block Design with three replications. Each replication had four treatments *i.e*. T_1 – GA_3@ 60 gm/ha, T_2- GA_3 @ 90gm/ha, T_3- GA_3 @120gm/ha and T_4- Control. Each treatment was accommodated in a plot size of 31.5 m^2 (1.75 m × 18 m) with 50-cm space kept between plot to plot and 1 meter between replications

Twenty one days old seedlings of UPRI 95-17A and UPRI 93-287 R were transplanted by following 6: 2 planting ratio for A and R, respectively. While transplanting the row spacing was kept 15 cm among the A lines, 20 cm among the A and R lines and 30 cm among the R lines. Each treatment/plot consists of 6 rows of A surrounded by 2 rows of Rat both sides with 18 cm length. Standard cultural practices were adopted to raise a normal healthy crop.

Treatment was given in two split doses at 10 per cent and 20 per cent heading stage by dividing GA_3 in two parts. Application was done as foliar spray by dissolving GA_3 in absolute alcohol and then making the spray solution in water while maintaining the concentration of GA_3 in ppm. Different concentrations were sprayed by Knapsac sprayer @ 500 litre of water per hectare. The experimental plot was isolated from other rice fields by distance of 100 meters. Besides distance isolation, physical barrier was also provided by planting daincha crop around the experimental field. Flag leaf clipping before foliar spray was done by cutting the 2/3 of the flag leave from top in order to expose the panicle for treatment and also to facilitate the free pollen movement. Supplementary pollination was done by shaking the pollen parents (R line) with the sticks between 9: 30 am to 11.30 am. This operation was done 4-5 times for a period of 10 days. The data in field was recoreded on five randomly selected plants from each plot of each replication for plant height (cm), number of tillers per plant, panicle length (cm), length of exerted panicle (cm), panicle exertion percent, number of spikelets per panicle, number of fertile spikelets per panicle, seed setting percent, 1000 grain weight (g) and grain yield per plant. Panicle exertion rate and seed setting percent was measured using the following formula

Panicle exertion percent = Length of exerted panicle/ Total length of panicle×100

Seed setting percentage = No. of fertile spikelets/ Total number of spikelets per panicle

The hybrid seed obtained from female parent was tested for germination and vigour traits. The data in laboratory was recorded for seed germination percent, shoot length (cm), root length (cm), seedling fresh weight (mg), seedling dry weight (mg), vigour index I and vigour index II. Vigour index I and vigour index II was measured using the following formula: -

Vigour index-I

Seedling Vigour Index-I= Standard germination percent x Seedling length (cm).

Vigour index-II

Seedling Vigour Index-II= Standard germination percent x Seedling dry weight (g).

The results were analysed using one way ANOVA for field data and two way ANOVA for laboratory data.

Result and discussion

Analysis of variance and means for effect of different doses of GA_3 application on female parental line.

1. Analysis of variance

In ordrer to partition the total variation into different components, mean values over replications for different treatment effects and for different characters were subjected to statistical analysis appropriate to the experimental design applied. The results pertaining to the analysis of variance are discussed here. Analysis of variance performed over four treatments for ten characters *viz*., plant height (cm), number of tillers per pl, panicle length (cm), length of exerted panicle (cm), panicle exertion percent, number of spikelets per panicle, number of filled spikelets per panicle, seed setting percentage, 1000 grain weight (g), seed yield per plot studied on female line (UPRI 95-17A). Results indicated highly significant mean square values of treatment difference for all the traits except number of spikelets per panicle and 1000 grain weight (g). This suggests that the treatment differed significantly in respect to their effect on different traits.

2. Analysis of means

The mean effect of four treatments of on ten characters studied for a line are presented in (Table 1). Mean values for plant height ranged from 73.33 (T_4) - 110.00 (T_3) cm with overall mean of 97.50 cm. Plant height was greatly influenced by GA_3 spraying. Height increase was in increasing order with the increase concentration of GA_3. Susilawati, *et al*. 2014 [8] also found that plant height greatly influenced by GA_3 spraying. The increase in plant height was due to increased activity of cells division, enlargement and elongation. Gibberellins are plant hormones that regulates various processes of plant growth and development, which is particularly important in stem elongation (Hedden and Phillips 2000; Sakamoto *et al.* 2004 and Tiwari *et al.* 2011) [15]. Mean values for number of tillers per plant ranged from 9.33 (T_4) -13.82 (T_3) with a general mean of 11.76 cm. Number of tillers per plant also increased significantly over control, in increasing order with the increased concentration of GA_3. But the intensity of increase was not high among different doses of GA_3. Niknejhad and Pirdashti 2012 [19] also reported that application of GA_3 have an enhancing effect on tillers number per plants. Values of mean for Panicle length (cm) varied from 19.66 (T_4) - 23.00 (T_2) with a general mean of 21.57. Length of exerted panicle (cm) was found to have mean values between 12.50 (T_4) - 20.66 (T_3), with a general mean of 17.31. Mean values of treatment effect for panicle exertion percent varied form 63.80 (T_4) - 89.90 (T_3), with a mean value of 79.69. Panicle length, length of exerted panicle (cm) and panicle exertion percent increased significantly with increase in GA_3 dose. **Tiwari *et al*. 2011[15].** Also found that spraying of GA_3 significantly increased the panicle length compared to control. This increase in length with increasing GA_3 level might be associated with stimulating effect of GA_3 on various physiological process including cell division and cell elongation in the plant Rahman *et al*. 2012[18]. Yin *et al* (2007) [16]. found that GA_3 affects the panicle base elongation of male sterile lines and panicle can be filled out from the flag

leaf sheath which enhances the opportunities of seed formation.

For number of spikelets per panicle the mean value ranged from 130.32 (T_4) - 136.32 (T_2), with a mean value of 133.89. No significant different was found among different treatments for number of spikelets per panicle. 1000 grain weight (g) had the mean value varied from 20.15 (T_3) - 21.67 (T_4), with a general mean of 20.66. For 1000 seed weight there was no difference among all the treatments. Nonsignificant difference among all the treatments for number of spikelets per panicle and 1000 grain weight (g) was also found by (Rahman *et al*. 2012 and Gavino *et al*. 2008) [2, 18]. which showed that 1000 grains weight was not affected by GA_3. It is a genetically controlled character and genetically fixed by an individual variety.

Number of filled spikelets per panicle was found to have mean values between 28.76 (T_4) – 49.41 (T_3), with a general mean of 41.59. Values of mean for Seed setting percentage varied from 22.06 (T_4) – 36.61 (T_3), with a general mean of 31.00. Like the other characters these characters also increased with the increased dose of GA_3. Seed setting per cent, the most important attribute in any hybrid crop was affected significantly with the different doses of GA_3 highest seed set per cent was recorded in T_4 (120g/ha). Although the GA_3 treatment applied at the lowest concentration (60g/ha) increased seed setting per cent greatlty over control. Beneficial effect of GA_3 application at various concentrations on grain per panicle and seed setting per cent was also earlier reported by (Rahman *et al*. 2012 and Gavino *et al*. 2008) [2, 18].For seed yield per plot (kg) the mean value ranged from 1.13 (T_4) - 2.10 (T_3), with a mean value of 1.77. Significant increase in seed yield per plot was obtained among all the treatments. Similar findings regarding increased seed yield as a result of GA_3 application has been reported by Susilawati, *et al.* (2014) and El-Degwy *et al.* (2010) [8, 5].

Like most other characters, this character also showed the increasing trend with increasing GA_3 concentration. Seed yield obtained with GA_3 dose 120g/ha (T_3) was found almost double in comparison to control (T_4). There was also significant diference was found for seed yield between control (T_4) and GA_3 dose 60g/ha (T_1). Difference for seed yield between GA_3 dose 60g/ha (T_1) and 90g/ha (T_2) was found *at par*.

Postharvest residual effect of GA_3 treatments on germination and vigour of hybrid seed.

Residual effect of GA_3 treatments application on germination and different seed vigour parameters *viz.*, Germination percent, shoot length (cm), root length (cm) fresh weight (mg) dry weight (mg), vigour index I and vigour index II was also tested on hybrid seed produced on A line, after different period of storage December (1 month), February (3 months), and April (5 months). Data pertaining to the germination and vigour parameters as influenced by different storage period (Factor A) and gibberellic acid treatments (Factor B) and their interaction (Factor A × Factor B) have been presented in (Table 2).

1. Response of storage period

Germination percentage was varied significantly with storage period. The highest germination percentage (91.583%) was recorded at the month of December, while lowest germination percentage (76.583%) was recorded at the month of April. The effect of storage period was also found significant for other seed vigour parameters *viz*., Shoot length (cm), root length (cm) fresh weight (mg) dry weight (mg), vigour index I and vigour index II. The highest shoot length (10.17cm) was found at month of December, while lowest (7.717) at month of April. The highest root length (10.392) was recorded at month of December and lowest (7.483) at month of April. The highest fresh weight and dry weight, 354.6 mg and 41.35 mg were observed at month of December, while lowest 265.18 mg and 28.23 mg, fresh weight and dry weight at month of April respectively. Vigour index I and vigour index II, 1885.0 and 3790.0 were recorded highest at month of December, while lowest 1166.9 and 2167 were observed at month of April respectively. Raikar, *et al*. (2011) [6] also reported the decrease in germination percent and seed vigour parameters with increase in storage period.

2. Response of GA_3 treatments

The effect of different GA_3 treatments was also found significant for the seed germination percentage and other seed vigour parameters *viz.*, shoot length (cm), root length (cm) fresh weight (mg) dry weight (mg), vigour index I and vigour index II.

The highest germination percentage (85.44%) was recorded for T_4 (control), while lowest germination percentage (80.55%) was recorded for T_3 (120g GA_3/ha). The highest shoot length (8.83 cm) and root length (8.81) were found for T_4 (control), while lowest (8.65) and (8.33 cm) for T_3 (120g GA_3/ha) respectively. Fresh weight and dry weight, 338.57 mg and 34.30 mg were found highest for T_4 (control), while lowest 293.45 mg and 33.291 mg in T_3 (120g GA_3/ha) respectively. The highest Vigour index I and Vigour index II, 1511.76, 2946.54 were recorded in T_4 (control), while lowest 1401.23, 2759.92 were observed in T_3 (120g GA_3/ha) respectively. Germination per cent and other vigour parameters of hybrid rice seed reduced significantly with the increased concentration of GA_3. Highest germination and vigour parameters were observed in T_4 (control) and lowest in T_3 (120g GA_3/ha). Similar findings regarding reduction in germination and seed vigour parameters in different GA_3 treatments were also observed by **Duan (1992)** [7]. **Ponnuswamy and Prabagaran (1997), Jagadeshwari *et al*. (1998) and Devi *et al*. (2014)** [13].

3. Interaction effect (storage period × GA_3 treatments)

The interaction effect of storage period and GA_3 treatments (Table 4.16) showed significant effect on seed germination per cent and seed vigour parameters *i.e*. Shoot length (cm), root length (cm) fresh weight (mg) dry weight (mg), vigour index I and vigour index II. Suggesting the greater impact of treatment application on these traits. Germination percent and seed vigour parameters showed the decreasing trend with the increasing dose of GA_3 and storage period. Germination per cent and seed vigour parameters were found higher among the GA_3 treatments as compared to control after 1 month storage. But, as the storage period increased, marked decrease in germination and vigour was observed in all the treatments. Lowest germination and seed vigour indices were observed in T_3 (120g GA_3/ha) at the end of storage period (April), while there was a gradual loss of germination and vigor in T_4 (control) in comparison to other GA_3 treatments followed by T_1 (60g GA_3/ha). Poor storability of the seeds of hybrid rice plants treated with GA_3 was also reported by Huang *et al.* (1997) [14]. Lowest germination in hybrid rice may be due to adverse effect of GA_3 application through increased alpha - amylase activity which reduced seed storability (Devi *et al.* 2014) [13].

Table 1: Mean values of treatment effects for ten characters in female parent UPRI 95-17A

Treatments	Characters									
	Plant height (cm)	Number of tillers per plant	Panicle length(cm)	Length of exerted panicle (cm)	Panicle exertion percent	Number of spikelets per panicle	Number of filled spikelets per panicle	Seed setting percentage	1000 grain weight (g)	Seed yield per plot (kg)
T_1 (60g GA_3/ha)	100.66	11.16	21.08	17.24	81.81	133.82	42.40	31.72	20.55	1.92
T_2 (90g GA_3/ha)	106.00	12.75	22.56	18.83	83.47	136.32	45.79	33.60	20.28	1.95
T_3 (120g GA_3/ha)	110.00	13.82	23.00	20.66	89.90	135.12	49.41	36.61	20.15	2.10
T_4 (Control)	73.33	9.33	19.66	12.50	63.80	130.32	28.76	22.06	21.67	1.13
Range	73.33-110.00	9.33-13.82	19.66-23.00	12.50-20.66	63.80-89.90	130.32-136.32	28.76-49.41	22.06-36.61	20.15-21.67	1.13-2.10
Gm	97.50	11.76	21.57	17.31	79.69	133.89	41.59	31.00	20.66	1.77
CD at 1%	7.46	2.29	1.25	1.75	10.40	8.34	4.91	5.03	2.21	0.17
CD at 5%	4.94	1.51	0.83	1.16	6.89	5.52	3.25	3.33	1.46	0.11
C.V.%	2.54	6.46	1.93	3.36	4.33	2.06	3.92	5.39	3.55	3.26

Table 2: Effect of storage period and GA_3 treatments on hybrid seed

Storage period/ GA3 dose	Characters														
	Germination (%)					Shoot length(cm)					Root length(cm)				
	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean
December (1months)	90.33	92.00	94.33	89.66	91.58	9.90	10.43	10.90	9.46	10.17	10.46	10.80	10.66	9.63	10.39
February (3months)	83.33	81.00	76.66	86.66	81.91	8.43	8.03	7.83	8.80	8.27	8.20	8.03	7.40	8.60	8.05
April (5 months)	81.00	74.66	70.66	80.00	76.58	7.80	7.60	7.23	8.23	7.71	7.56	7.23	6.93	8.20	7.48
MEAN	84.88	82.55	80.55	85.44		8.71	8.68	8.65	8.83		8.74	8.68	8.33	8.81	
	Factor A*	Factor B*	AXB*			Factor A*	Factor B*	AXB			Factor A*	Factor B*	AXB*		
SEM	0.259	0.299	0.518			0.027	0.031	0.054			0.063	0.072	0.125		
CD at 5%	0.761	0.878	1.521			0.079	0.091	0.157			0.184	0.212	0.367		

Table 3

Storage period/GA_3 dose	Characters														
	Fresh weight(mg)					Dry weight(mg)					Vigour indexI				
	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	Mean
December (1months)	351.26	358.10	362.16	347.03	354.64	40.63	41.86	43.90	39.00	41.35	1,839.80	1,953.33	2,034.56	1,712.60	1,885.07
February (3months)	332.10	326.0	304.10	340.20	325.61	32.36	32.36	30.46	34.37	32.39	1,386.16	1,301.40	1,168.00	1,508.00	1,340.89
April (5 months)	296.00	222.13	214.10	328.50	265.18	28.50	29.40	25.50	29.53	28.23	1,244.53	1,107.60	1,001.13	1,314.70	1,166.99
MEAN	326.45	302.10	293.45	338.57		33.83	34.54	33.29	34.303		1490.16	1454.11	1401.23	1511.76	
	Factor A*	Factor B*	Axb*			Factor A*	Factor B*	Axb*			Factor A*	Factor B*	Axb*		
SEM	0.510	0.589	1.021			0.239	0.276	0.478			8.359	9.652	16.718		
CD at 5%	1.499	1.731	2.99			0.701	0.810	1.403			24.544	28.341	49.088		

Table 4

Storage period/ GA_3 dose	Character				
	Vigour indexII				
	T_1 (60g GA_3/ha)	T_2 (90 g GA_3 /ha)	T_3 (120 g GA_3/ha)	T_4 (Control)	MEAN
December (1months)	3,670.5	3,851.3	4,141.1	3,497.16	3790.03
February (3 months)	2,697.1	2,622.0	2,335.5	2,979.70	2658.6
April (5 months)	2,308.6	2,195.6	1,803.1	2,362.77	2167.5
MEAN	2,892.0	2,889.6	2759.9	2946.5	
	FACTOR A*	FACTOR B*	AXB*		
SEM	22.69	26.208	45.394		
CD at 5%	66.64	76.95	133.289		

Conclusion

Seed yield obtained with GA_3 dose 120g/ha (T_3) was found almost double in comparison to control (T_4). The significant diference was found for seed yield between control (T_4) and GA_3 dose 60g/ha (T_1). Difference for seed yield between GA_3 dose 60g/ha (T_1) and 90g/ha (T_2) was found *at par*.

More yield can be obtained by using GA_3 dose 120g/ha (T_3). But, it will not be economically viable and high dose of GA_3 also adversaly affects the seed germination and vigour during storage. While, GA_3 dose 60g/ha (T_1) also gave the significant yield increase over control and gradual loss of germination and vigour was also observed in T_1 (60g GA_3/ha) in comparison to other GA_3 treatments. So, from the economic point of view, GA_3 dose 60g/ha (T_1) can be recommended to obtain good seed yield and minimize the cost of input in the hybrid rice seed production.

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