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Improvement of parental lines' performance by spraying cytokinin and molybdenum on hybrid rice seed production

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ABSTRACT

Field investigations were carried out at the Experimental Farm, Rice Research and Training Center, Field Crops Institute, and ARC during the growing seasons 2021 and 2022 to study the effect of cytokinin and molybdenum on hybrid seed production. The experiments comprised four treatments, viz., T1 (control), T2 (cytokinin) with a 20 ppm concentration, T3 (molybdenum) with a 35 ppm concentration, and T4 (a 1:1 mixture of cytokinin 10 ppm and molybdenum 17.5 ppm) as a pre-flowering treatment for male parent Giza 178 R and female parent for five Cytoplasmic Male Sterility (CMS) lines (IR69625, IR70368, IR58025, K17, and G46) on hybrid rice seed production. The treatments were conducted. The CMS lines were arranged in the main plots, while treatment applications were distributed in subplots and three replications. The exogenous application of treatments such as cytokinin and molybdenum could improve the crossing rates of the male parent by affecting floral traits and, accordingly, increase hybrid rice seed production. The most significant effects of treatment on anther length, anther width, pollen fertility, number fertility, stigma width, stigma length, days to heading, number of fertile tiller hills-1, plant height, panicle length, seed set, and grain yield of male parent Giza 178R were (T4), a combination between cytokinin and molybdenum. Duration of floret opining, total stigma length, stigma length, angle of floret opining, stigma width, stigma burch, days to heading, plant height, number of fertile panicles hill-1, flag leaf angle, panicle exertion, panicle length, panicle mass, seed set, seed yield, and harvest index of CMS lines were significantly affected by treatments. Moreover, the evaluated CMS lines exhibited significant differences in all measured floral traits. Line 2, Line 3, and Line 1 displayed the uppermost spikelet opening angle, duration of spikelet opening, total stigma length, style length, stigma brush, and stigma width. In addition, these CMS lines exhibited the highest plant growth and yield traits, particularly under T4. Consequently, exogenous application of T4 in combination with cytokinin and molybdenum could be exploited to improve the floral, growth, and yield traits of promising CMS lines such as Line 1, Line 3, and Line 4, thereby increasing outcrossing rates and hybrid rice seed production.

Keywords: Hybrid rice production; out cross enhancing; Cytokinin; Molybdenum.

INTRODUCTION

Hybrid rice is one of the most important ways to increase rice production, Hybrid rice (HR) seed production is a profitable venture offer extra income (Rs. 75000-85000/ha net return) and has additional employment opportunity to~20.0 million rurals (requires 100-105 extra man days'/ha area of HR seed production). Therefore, this entity has great scope for improvement of livelihood of the nation (Rout *et al.,* 2020). Given its yield advantage and economic importance, several hybrids in rice have been commercialized in more than 40 countries including Egypt, which creates a huge seed industry world-wide (Verma *et al.,* 2018). The three-line system of seed production involving CMS, maintainer and restorer lines that is being commonly used for large scale hybrid rice seed production in the world. The two-line approach involving environmental sensitive genetic male sterility is also being practiced in many countries for successful hybrid rice seed production (Tanweer *et al.,* 2020). The drawback of the three-line method is the low seed production, mainly due to poor panicle exertion and the low outcrossing rate. Enhancing outcrossing rates in the CMS lines considerably increases hybrid seed production. Plant growth regulators could be used as exogenous applications to enhance hybrid rice seed production. Several earlier reports elucidated that the exogenous application of plant growth regulators improved hybrid rice seed production. In this context, Tiwari *et al.,* (2011) demonstrated that GA3 followed by Cytokinin (CK), NAA, urea, and K2PO4 enhanced hybrid rice seed production. Similarly, Pan *et al.,* (2013) deduced that spraying 50 mg/L paclobutrazol followed by 30 mg/L 6-benzyl amino purine and gibberellic acid at the heading stage increased the number of spikelets/panicles, seed setting rate, and rice grain yield.

Cytokinins (CKs) are a class of plant hormones known as key regulators of plant growth and development, including cell division, chloroplast biogenesis, bud and root differentiation, shoot meristem initiation and growth, stress tolerance, and organ senescence (Argueso *et al.,* 2009 andKuroha *et al.,* 2009). Appropriate modulation of the activity of phytohormones, which are crucial regulators of growth and development, can substantially contribute to increasing yield of this and other cereal crops. One such phytohormone, cytokinin, is a particularly promising target for improving crop species (Jameson and Song, 2016) as it regulates nearly all plant processes, many of which have agronomic relevance, including meristem activity, leaf senescence, nutrient uptake, various abiotic and biotic interactions, and multiple developmental pathways (Kieber and Schaller, 2014, 2018; Christian *et al.,* 2020).

Molybdenum (Mo) is present as a pterin-cofactor in the active center of plant enzymes catalyzing key steps of nitrogen, carbon, and sulfurmetabolisms, making them essential for efficient growth under the diverse environmental conditions. The importance of Mo for plant growth and development, together with its scarcity in soils, make necessary accurate homeostatic machinery in order to coordinate cellular Mo demand and metal availability. Although basic principles of Mocofactor biosynthesis are understood, our knowledge of other key processes of plant Mo homeostasis, such as Mo uptake and transport or Mo storage, is very limited. The Mo cofactor biosynthesis, and the role played by the molybdoenzymes in plant biology are addressed. Recent studies have pointed the great importance of Molybdenum (Mo) to wheat at physiological and molecular levels (Al-Issawi *et al.,* 2016, Al- Issawi *et al.,* 2013; Babenko and Alikulov, 2014). Mo is inactive in biological system if not combined with special cofactors (Rihan *et al.,* 2014, Sun *et al.,* 2009). It found to be co-factor in more than forty molybdoenzymes in all organisms, however it found only in four enzymes in plants namely nitrate reductase (NR), xanthin dehydrogenase (XDH), sulphate oxide (SO) and aldehyde oxidase (AO) (Mendel and Hänsch, 2002) which participate in many metabolic reactions; mainly assimilation of nitrate, phytohormone synthesis, purine catabolism and sulphate detoxification in plants (Mendel and Hänsch, 2002, Mendel and Schwarz, 1999).Thereupon, the present study aimed at exploring the influence of Cytokinin (Ck) and molbdynum (Mo) on floral traits and hybrid rice seed production. Moreover, to assess the performance of diverse rice cytoplasmic male sterile lines under the application of used growth regulators.

MATERIALS AND METHODS

Experimental Site and Agricultural Treatments:

Field experimentation was performed at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt (30◦570 N, 31◦070 E) during the summer of 2021 and 2022. The experimental site is characterized as a hot and arid climate with no precipitation during the summer season. The soil is old Nile valley clay throughout the profile (12.6% sand, 32.4% silt, and 55% clay). Organic matter, pH, and electrical conductivity were 1.39 g/kg, 8.1, and 3.30 dS/m, respectively. Soluble cations and anions were 4.3, 1.88, 16, 4.55, and 5.55 mmolc/L for Mg2+, Na+, K+, Fe3+, and HCO3−, respectively. The experimental design was a split plot with three replications. The CMS lines ('IR69625, IR70368, IR58025, K17 and 'G46') were applied in the main plots, whereas the exogenous foliar applications of growth regulators were randomized in sub-plots. An isolation space of 100 m was considered for CMS seed production. Moreover, the experimental field was surrounded by an additional 20 rows of R lines to avoid any possibility of cross-pollination. Every main plot was isolated by a plastic barrier (2.5 m height) to avoid any pollen grain movement from one treatment to another. The nursery seedbed was well plowed and dry-leveled. Phosphorous fertilizer was applied at a rate of 36 kg P2O5/ha as super-phosphate (15.5% P2O5) and potassium at a rate of 48 kg K2O kg/ha as potassium sulfate (48% K2O) before tillage. Nitrogen at the rate of 165 kg N/ha in the form of urea (46% N) was added in two splits: one-third as basal dressing and the rest at panicle initiation. Rice seeds at a rate of 20 kg/ha (15 kg of CMS line and 5 kg of the restorer line) were soaked in freshwater for 24 h, then drained and incubated for 48 h to hasten early germination. The seeds of each line were sown in the nursery on the 7 of May in both seasons. At the age of 30 days, seedlings were hand transplanted into hills with a row ratio of 2R:10A, 0.30 m between B line rows, 0.15 m between A line rows, 0.20 m between B and A lines, and 0.15 m between hills (A and R lines) with two seedlings per hill and each row was 5 m long. The row direction was perpendicular to the wind direction, and supplementary pollination was carried out artificially by shaking the pollen parent's canopy with a stick during flowering to spread the pollen grains of A lines. The operation of shaking the pollen parent's canopy was done three times between 9 and 11.30 a.m. at 30 min intervals for a period of 10 days.

Plant Material and Foliar Application:

Five CMS lines were used in this study, including three from the International Rice Research Institute (IRRI) and Gambiaca and Kalinga from China, which were selected for their genetic differences (Table 1). Three foliar applications were applied: 1)- Cytokinin (CK, 20ppm), 2)- Molybdenum (MO 35ppm) and 3)- combination of Cytokines (CK, 10ppm) and Molybdenum (MO17.5ppm) mixture versus untreated control. The Cytokinin and

Molybdenum were purchased from Sigma and it applied in two times; first spray (when A and R line was at 15-20 % heading and the second spray was applied when A and R line was at 35-40 % heading, which, (five days after heading) for male parent Giza 178 R and female parent for five Cytoplasm Male Sterility (CMS) lines (IR69625, IR70368, IR58025, K17 and G46) on hybrid rice seed production. Cytokinin and Molybdenum were dissolved in amount of 70% ethanol alcohol, combined with 50 L of water, and sprayed.

Table 1. Cytoplasmic male sterile (CMS) lines used for the study.

Code	Genotype	Days to Heading	Source	Origin
L1	IR69625A	105.5	Wild abortive (WA) CMS line	IRRI
L2	IR58025A	108.2	Wild abortive (WA) CMS line	IRRI
L3	IR70368A	103.3	Wild abortive (WA) CMS line	IRRI
L4	G46A	88.9	Gambiaca CMS line	China
L5	K17A	84.5	Kalinga type	China
178 R	Giza 178 R	100.7	Giza175/Milyang 49 Indica/Japonica	Egypt

Measured Traits Data were recorded from five randomly selected hills excluding border rows per sub-plot for the studied characters. The floral traits were measured following Singh and Haque [46] using a micrometer under a stereomicroscope. The studied floral traits were duration of spikelet opening (min), spikelet opening angle (◦), total stigma length (mm), style length (mm), stigma brush (mm), and stigma width (mm). Moreover, growth and yield traits were recorded for ten randomly selected plants from each plot. Plant height (cm), flag leaf angle (O), number of fertile tillers per hill, panicle length (cm), panicle exertion (%), panicle weight (g), seed set (%), grain yield (ton ha^{-1}), and harvest index (%) were recorded. The crop was harvested when 85% of the grains became golden yellow. Grains were sun-dried and adjusted at 14% moisture content to estimate grain yield.

Panicle exertion percentage was estimated according to the following formula:

$$\text{Panicle exsertion \%} = \frac{\text{Exserted panicle length (cm)}}{\text{Panicle length (cm)}} \times 100$$

Seed set percentage was calculated according to the following formula:

$$\text{Seed set \%} = \frac{\text{Number of filled grains/panicle}}{\text{Total Spikelet number/panicle}} \times 100$$

STATISTICAL ANALYSIS

All data collected were subjected to standard statistical analysis of variance following the method described by Gomez and Gomez (1984). Different means were compared by Duncan's multiple range test (DMRT) with a 5 % probability level.

RESULTS

Effect of Cytokinin and Molybdenum application on male parent:

The effect of Cytokinin and Molybdenumapplication on male parent traits such anther length, anther width, pollen fertility, number pollen fertility, stigma width, stigma length, days to heading, number of fertile tiller $hill^{-1}$, plant height, panicle length, seed set and grain yield of male parent Giza 178R, are presented in (Table 2). The results indicated that, Cytokinin and Molybdenumapplied for male parent recorded a significant increase ofanther length, anther width, pollen fertility, number fertility, stigma width, stigma length, days to heading, number of fertile tiller $hill^{-1}$, plant height, panicle length, seed set and grain yield of male parent Giza 178R as compared with control treatment in both seasons. Application of combination between Cytokinin and Molybdenumon male parent gave the highest anther length (2.38 and 2.40 mm), (0.53 and 0.56 mm) for anther width , the highest values of pollen fertility (96.88 and 97.72 %), number fertility (1820.66 and 1849.70), stigma width (0.48 and 0.51 mm),stigma length (0.42 and 0.44mm), days to heading (98.12 and 97.71), number of fertile tiller $hill^{-1}$ (20.72 and 23.60), (124.26 and 128.86), panicle length (24.81 and 25.21cm), seed set (96.32 and 95.55 %) and grain yield (9.384 and 9.684 t ha^{-1}) during 2021 and 2022 seasons, respectively. But, the lowest values obtained with untreated Cytokinin and Molybdenum of anther length (2.23 and 2.25 mm), (0.46 and 0.50 mm) for anther width , the highest values of pollen fertility (93.21 and 94.05 %), number fertility (1710.60 and 1739.65), stigma width (0.35 and 0.36 mm), stigma length (0.31 and 0.34mm), days to heading (96.91 and 95.71), number of fertile tiller $hill^{-1}$ (13.58 and 15.48), (94.51 and 98.62), panicle length (21.11 and21.50cm), seed set (92.98 and 92.54 %) and grain yield (7.884 and 7.984 t ha^{-1}) during 2021 and 2022 seasons, respectively.

Table 2. Floral traits, growth characters and yield component of male parent (Giza 178) as affected by Cytokinin and Molybdenum application rates during 2021 and 2022 growing seasons.

Main effect and interaction	AL(mm)		AW (mm)		PF (%)		NFP		SW (mm)		SL(mm)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1	2.23d	2.25d	0.46c	0.50c	93.21c	94.05c	1710.6d	1739.6d	0.35d	0.36c	0.31c	0.34c
T2	2.31c	2.33c	0.51b	0.55b	93.54c	94.38c	1769.0c	1798.0c	0.44c	0.47b	0.38b	0.41b
T3	2.34b	2.37b	0.52b	0.55b	95.21b	96.05b	1794.0b	1823.0b	0.45b	0.47b	0.42a	0.44a
T4	2.38a	2.40a	0.53a	0.56a	96.88a	97.72a	1820.6a	1849.7a	0.48a	0.51a	0.42a	0.44a
F-test	**	**	**	*	**	**	**	**	**	**	**	**

AL: Anther length, AW: Anther width, PF: Pollen fertility, NFP: Number of fertile pollens, SW: Stigma width, SL: Stigma length.

Contented Table 2.

Main effect and interaction	DTH (day)		NFT		PH (cm)		PL(cm)		SS (%)		SY (t ha^{-1})	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1	96.91c	95.71c	13.58d	15.48d	94.51d	98.62d	21.11d	21.50d	92.98c	92.54c	6.884d	7.184d
T2	98.12a	97.91a	18.89b	20.79b	112.66b	117.52b	23.76b	24.16b	94.68b	93.72b	7.083c	7.383c
T3	97.96b	96.84b	16.22c	18.12c	103.17c	108.00c	22.27c	22.67c	95.86a	94.76a	7.217b	7.517b
T4	99.11a	97.71a	20.72a	23.60a	124.26a	128.86a	24.81a	25.21a	96.32a	95.55a	7.384a	7.684a
F-test	**	**	**	**	**	**	**	**	**	**	**	**

DTH: Days to heading, NFT: Number of fertile tillers, PH: Plant height, PN: Panicle length, SS: Seed set, SY: Seed yield.

F1 seeds of CMS lines characteristic:

CMS lines characteristic as affected by Cytokinin and Molybdenumapplication on floral traitsas, duration of floret opining, total stigma length, stigma length, angle of floret opining, stigma width, stigma Burch were highly significantly affected by Cytokinin and Molybdenumapplication rates of CMS (Table 3).

Floral traits of CMS lines:

The results showed that there were significant differences between the CMS lines (' IR69625, IR58025, IR70368, G46 and K17').L3 gave the longest duration of floret opining (DFO) (139.28 and 154.28 min), the highest values of angle flowering opining (AFO) (32.34 and 33.24%) recorded with L4, L2 gave the highest values (2.32 and 2.35 mm) of total stigma length (TSL) and (1.06 and 1.11 mm) of stigma length(SL), while, L1 recorded the highest values (0.62 and 0.64 mm) of stigma width (SW) and L5 recorded the highest values (1.42 and 1.44 mm)of stigma Burch (SB) in both seasons. On the other hand L2 gave the lowest duration of floret opining (DFO)(93.71 and 108.71min), (25.78 and 26.68) of angle of floret opining (AFO), L1 gave the least values (2.14 and 2.17 mm) of total stigma length (TSL), L4 recorded the least values (0.57 and 0.67 mm) of stigma length (SL), L5 gave the lowest values (0.48 and 0.52 mm)of stigma width (SW) and L3 recorded the least values(1.23 and 1.24 mm) of stigma Burch (SB) during the both seasons.

Floral traits affect by foliar treatments:

The results showed that, there were significant differences among treatments on duration of floret opining (DFO), angle of floret opining (AFO), total stigma length (TSL), stigma length (SL), stigma width (SW), stigma Burch(SB)in both seasons. T4recorded the highest values (142.25 and 157.25 min) of duration of floret opining (DFO), (33.71 and 34.62 %) of angle of floret opining (AFO), (2.61 and 2.35 mm) of total stigma length (TSL), (0.87 and 0.94 mm) of stigma length(SL), (0.63 and 0.66 mm) of stigma width (SW) and (1.42 and 1.45 mm) of stigma Burch (SB) in both seasons. On the other handT1 (without application)recorded the lowest values (104.74 and 119.70 min) of duration of floret opining (DFO), (24.98 and 25.88 %) of angle of floret opining (AFO), (1.80 and 1.83 mm) oftotal stigma length (TSL), (0.82 and 0.88 mm) of stigma length (SL), (0.62 and 0.64 mm) of stigma width (SW) and (1.24 and 1.26 mm) of stigma Burch (SB) during the both seasons.

Interaction effects:

the interaction highly significantly effected on duration of floret opining (DFO), angle of floret opining (AFO), total stigma length (TSL), stigma length (SL), stigma width (SW), stigma Burch(SB) in both growing seasons.

Table 3. Influence of Cytokinin and Molybdenumon floral traits during 2021 and 2022 growing of CMS lines.

Main effect and interaction	DFO (min)		AFO (%)		TSL (mm)		SL (mm)		SW(mm)		SB(mm)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
CMS lines (L)												
L1 (A1 × G178R)	126.49c	141.49c	31.60a	32.50a	2.14d	2.17d	0.82b	0.88b	0.62a	0.64a	1.24c	1.26c
L2 (A2 × G178R)	93.71e	108.71e	25.78d	26.68d	2.32a	2.35a	1.06a	1.11a	0.50d	0.53d	1.32b	1.33b
L3 (A3 × G178R)	139.28a	154.28a	27.41c	28.31c	2.31a	2.33a	0.76c	0.83c	0.55b	0.58b	1.23d	1.24d
L4 (A4 × G178R)	122.22d	137.23d	32.34a	33.24a	2.18c	2.21c	0.57e	0.67e	0.53c	0.56c	1.25c	1.27c
L5 (A5 × G178R)	133.16b	148.16b	29.50b	30.40b	2.21b	2.24b	0.66d	0.73d	0.48d	0.52d	1.42a	1.44a
F-test	**	**	**	**	**	**	**	**	**	**	**	**
Treatment (T)					9							
T1	104.74d	119.74d	24.98d	25.88d	1.80d	1.83d	0.67d	0.75d	0.43d	0.46d	1.15d	1.16d
T2	117.15c	132.15c	28.19c	29.09c	2.14c	2.16c	0.74c	0.80c	0.51c	0.54c	1.25c	1.27c
T3	127.75b	142.75b	30.41b	31.31b	2.37b	2.41b	0.82b	0.88b	0.57b	0.61b	1.34b	1.36b
T4	142.25a	157.25a	33.71a	34.62a	2.61a	2.64a	0.87a	0.94a	0.63a	0.66a	1.42a	1.45a
F-test	**	**	**	**	**	**	**	**	**	**	**	**
LxT	**	**	*	*	**	**	**	**	**	**	**	**

DFO: Duration of floret opining, AFO: Angle of floret opining, TSL: Total stigma length, SL: Stigma length, SW; Stigma width, SBL: Stigma brush length.

The results in **Table (4)** indicated that, the interaction between the CMS lines and floral application were highly significantly affected on duration of floret opining (DFO), angle of floret opining (AFO), total stigma length (TSL), stigma length (SL), stigma width (SW), stigma Burch(SB) in both seasons. The CMS line IR58025A (L3) gave the longest duration of floret opining (DFO) (153.83 and 168.80 min) recorded under combination Cytokinin and Molybdenum(T4), the CMS (L4) gave highest values of angle flowering opining (AFO) (36.52 and 37.42) recorded with combination Cytokinin and Molybdenum(T4), L2 gave the highest values (2.80 and 2.83 mm) of total stigma length (TSL) with application combination Cytokinin and Molybdenum(T4) and (1.15 and 1.18 mm) of stigma length (SL) when application combination Cytokinin and Molybdenum(T4), while, L1 recorded the highest values (0.67 and 0.71 mm) of stigma width (SW) with combination Cytokinin and Molybdenum (T4) and L5 recorded the highest values (1.55 and 1.56 mm) of stigma Burch (SB) with combination Cytokinin and Molybdenum(T4) in both seasons. On the other hand the CMS (L2) gave the lowest duration of floret opining (DFO) (73.75 and 88.70 min) and (21.60.78 and 22.50) of angle of floret opining (AFO) without application (T1) , the CMS (L1) gave the least values (1.54 and 1.57 mm) of total stigma length (TSL) and (1.08 and 1.10mm) of stigma Bruch (SB) without application (T1), the CMS (L4) recorded the least values (0.53 and 0.63 mm) of stigma length (SL) and (0.36 and 0.39 mm) of stigma width (SW) without application (T1) during the both seasons.

Table 4. Effect of interaction between CMS liens and floralapplication of CMS lines during 2021 and 2022 seasons

Interaction		DFO (min)		AFO (%)		TSL (mm)		SL (mm)		SW (mm)		SB (mm)	
		2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
L1 (A1 × G178R)	**T1**	111.50j	113.70k	27.84e-h	28.74e-h	1.54p	1.57p	0.74j	0.75k	0.54fg	0.56fg	1.08l	1.10l
	T2	120.16h	135.14h	30.12cd	31.02cd	2.07j	2.10j	0.79h	0.89h	0.61d	0.63d	1.22j	1.25j
	T3	142.76c	157.66c	33.78b	34.68b	2.39e	2.42e	0.85g	0.92f	0.65b	0.68b	1.31g	1.32g
	T4	144.33bc	159.30bc	34.66ab	35.54ab	2.57c	2.60c	0.88f	0.95d	0.67a	0.71a	1.36e	1.38e
L2 (A2 × G178R)	**T1**	73.75n	88.70n	21.60l	22.55l	1.97l	2.00l	0.96d	1.06c	0.41l	0.44l	1.12k	1.13k
	T2	84.89m	99.83m	25.05ijk	25.95ijk	2.11i	2.14i	1.01c	1.11b	0.48i	0.51i	1.24i	1.26i
	T3	95.20l	110.22l	26.66ghi	27.56ghi	2.40e	2.43e	1.13b	1.12b	0.51gh	0.54gh	1.36e	1.37e
	T4	121.00h	136.00h	29.80cde	30.70cde	2.80a	2.83a	1.15a	1.18a	0.61d	0.63d	1.50b	1.53b
L3 (A3 × G178R)	**T1**	124.75g	139.85g	23.66k	24.76k	1.85n	1.88n	0.62m	0.72m	0.43jk	0.46jk	1.11k	1.13k
	T2	134.21e	149.22e	26.12hij	27.02hij	2.30g	2.33g	0.71k	0.74l	0.54fgh	0.55fgh	1.21j	1.23j
	T3	146.16b	161.18b	28.08e-h	28.98e-h	2.44d	2.47d	0.80h	0.91g	0.63cd	0.65cd	1.26h	1.29h
	T4	153.83a	168.80a	31.77c	32.57c	2.63b	2.66b	0.92e	0.94e	0.64bc	0.66bc	1.33f	1.35f
L4 (A4 × G178R)	**T1**	111.50j	126.51j	27.13fgh	28.03fgh	1.78o	1.81o	0.53p	0.63o	0.42kl	0.45kl	1.11k	1.13k
	T2	117.00i	131.98i	31.08c	31.02cd	2.19h	2.22h	0.55o	0.63o	0.49h	0.53hi	1.22j	1.24j
	T3	120.33h	135.30h	34.64ab	35.44ab	2.28g	2.31g	0.58n	0.68n	0.57e	0.60e	1.31g	1.34g
	T4	140.10d	155.09d	36.52a	37.42a	2.46d	2.49d	0.64l	0.74l	0.65bc	0.67bc	1.35e	1.38e
L5 (A5 × G178R)	**T1**	115.00i	130.00i	24.60jk	25.56j	1.89m	1.82m	0.53p	0.63o	0.36m	0.39m	1.31g	1.34g
	T2	129.50f	144.48f	28.62d-g	29.52d-g	2.01k2.	2.04k	0.61m	0.63o	0.45j	0.48j	1.41d	1.42d
	T3	134.31e	149.32e	28.88def	29.78def	2.36f	2.39f	0.71k	0.81j	0.54f	0.56f	1.44c	1.46c
	T4	152.00a	167.00a	35.83a	36.73a	2.59c	2.62c	0.78i	0.88i	0.61d	0.63d	1.55a	1.56a

DFO: Duration of floret opining, AFO: Angle of floret opining, TSL: Total stigma length, SL: Stigma length, SW; Stigma width, SBL: Stigma brush length.

F1 seeds of CMS lines characteristic Were highly significantly affected by Cytokinin and Molybdenum application rates for CMS lines on growth and yield component as, days to heading (DH), plant height (PH), number of fertile panicles hill^{-1} (NFPH), flag leaf angle (FLA), panicle exsertion (PE), panicle length (PL), panicle mass (PM), seed set (SS), harvest index (HI) and seed yield (SY) (Table 5).

CMS lines affect on growth characters and yield component:

The results showed that there were significant differences between the CMS lines (, IR69625, IR58025, IR70368, G46 and K17,). The CMS (L2) recorded the highest with all traits, (110.14 and 108.94 day) of days to heading (DH) , (108.04 and 109.88 cm) of plant height (PH), (16.98 and 18.98 hill^{-1}) of number of fertile panicles hill^{-1} (NFPH), (33.62 and 35.12^0) of flag leaf angle (FLA), (64.93 and 67.53 %) of panicle exsertion (PE), (22.97 and 23.04cm) of panicle length (PL), (2.53 and 2.61g) of panicle mass (PM), (20.32 and 22.92%) of seed set (SS), (17.33 and 18.22 mm) of harvest index (HI) and (1.571 and 1.630t ha^{-1}) of seed yield (SY) in both seasons. But, the least values for all traits obtained with the CMS (L5) (89.11 and 87.91 day) of days to heading (DH) , (98.74 and 100.48 cm) of plant height (PH), (13.71 and 15.48 hill^{-1}) of number of fertile panicles hill^{-1} (NFPH), (27.86 and 29.36^0) of flag leaf angle (FLA), (62.22 and 65.00 %) of panicle exsertion (PE), (20.54 and 20.61 cm) of panicle length (PL), (1.96 and 2.05 g) of panicle mass (PM), (19.76 and 22.36 %) of seed set (SS), (16.22 and 17.12 mm) of harvest index (HI) and (1.348 and 1.407 t ha^{-1}) of seed yield (SY) in both seasons.

Table 5. Influence of Cytokinin and Molybdenumon growth characters and yield component of during 2021 and 2022 growing of CMS lines.

Main effect and interaction	DTH (day)		PH (cm)		NFPH		FLA (^{0})		PE (%)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
CMS lines (L)										
L1 (A1 × G178R)	106.13b	105.01b	100.02c	101.86c	14.32b	16.02b	29.58c	30.98c	62.79b	65.37b
L2 (A2 × G178R)	110.14a	108.94a	108.04a	109.88a	16.98a	18.68a	33.62a	35.12a	64.93a	67.53a
L3 (A3 × G178R)	105.43b	104.23b	106.83ab	108.67ab	14.51b	16.21b	32.32b	33.83b	65.42a	67.98a
L4 (A4 × G178R)	90.33c	89.13c	106.62b	108.46b	14.76b	16.46b	32.57b	34.07b	63.42b	65.96b
L5 (A5 × G178R)	89.11c	87.91c	98.74d	100.48d	13.71c	15.48c	27.86d	29.36d		65.00b
F-test	**	**	**	**	**	**	**	**	**	**
Treatment (T)										
T1	98.91c	97.41c	89.81d	91.67d	11.08d	12.84d	26.26d	27.68d	51.35d	53.93d
T2	99.97b	97.71c	108.06b	109.91b	16.39b	18.09b	32.34b	33.83b	65.47b	68.07b
T3	100.90a	98.84b	98.57c	100.42c	13.72c	15.42c	29.63c	31.14c	58.06c	60.66c
T4	101.12a	99.92a	119.66a	121.51a	18.22a	19.92a	36.55a	38.05a	80.14a	82.74a
F-test	**	**	**	**	**	**	**	**	**	**
LxT	*	*	**	**	*	*	**	**	**	**

DTH: Days to heading, PH: Plant height, NFPH: Number of fertile panicles hill-1, FLA: Flag leaf angle, PE: Panicle exsertion

Treatments effect on growth characters and yield component:

The results showed that there were significant differences between application Cytokinin and Molybdenumon the CMS lines (, IR69625, IR58025, IR70368, G46 and K17,). Combination between Cytokinin and Molybdenum(T4) recorded the highest with all traits, (101.12 and 99.92 day) of days to heading (DH) , (119.66 and 121.51 cm) of plant height (PH), (18.22 and 19.92 hill^{-1}) of number of fertile panicles hill^{-1} (NFPH), (36.55 and 38.05^0) of flag leaf angle (FLA), (80.14 and 82.74 %) of panicle exsertion (PE), (23.61and 23.68 cm) of panicle length (PL), (2.87 and 2.95 g) of panicle mass (PM), (27.46 and 30.06 %) of seed set (SS), (19.74 and 20.64%) of harvest index (HI) and (1.838 and 1.897 t ha^{-1}) of seed yield (SY) in both seasons. But, the least values for all traits obtained without application (T1) (98.91 and 97.11 day) of days to heading (DH) , (89.81 and 91.67 cm) of plant height (PH), (11.08 and 12.48 hill^{-1}) of number of fertile panicles hill^{-1} (NFPH), (26.26 and 27.68^0) of flag leaf angle (FLA), (51.35 and 53.93 %) of panicle exsertion (PE), (19.91 and 19.94 cm) of panicle length (PL), (1.25 and 1.29 g) of panicle mass (PM), (8.93 and 11.53 %) of seed set (SS), (12.88 and 13.78%) of harvest index (HI) and (0.727 and 0.786 t ha^{-1}) of seed yield (SY) in both seasons.

The interaction highly significantly affected on days to heading (DH), plant height (PH), number of fertile panicles hill^{-1} (NFPH), flag leaf angle (FLA), panicle exsertion (PE), panicle length (PL), panicle mass (PM), seed set (SS), harvest index (HI) and seed yield (SY) (Table 5) in both seasons.

Contented Table.5

	PL (cm)		PM (g)		SS (%)		HI (%)		SY (t ha^{-1})	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
CMS lines (L)										
L1 (A1 × G178R)	21.39ab	21.42ab	1.89e	1.92e	18.21c	20.80c	17.85a	18.74a	1.470b	1.529b
L2 (A2 × G178R)	22.97a	23.04a	2.53a	2.61a	20.32a	22.92a	17.33a	18.22a	1.571a	1.630a
L3 (A3 × G178R)	22.91a	22.97a	2.30b	2.42b	19.46b	22.06b	17.26ab	18.16ab	1.454c	1.513c
L4 (A4 × G178R)	21.14ab	21.21ab	2.15c	2.26c	18.34c	20.94c	16.34bc	17.24bc	1.369d	1.428d
L5 (A5 × G178R)	20.54b	20.61b	1.96d	2.05d	19.76ab	22.36ab	16.22c	17.12c	1.348e	1.407e
F-test	**	**	**	**	**	**	**	**	**	**
Treatment (T)										
T1	19.91d	19.94d	1.25d	1.29d	8.93d	11.53d	12.88d	13.78d	0.727d	0.786d
T2	22.56b	22.63b	2.33b	2.19c	17.38c	19.98c	16.77c	17.67c	1.504c	1.563c
T3	21.07c	21.14c	2.33b	2.58b	23.09b	25.69b	18.59b	19.48b	1.702b	1.761b
T4	23.61a	23.68a	2.87a	2.95a	27.46a	30.06a	19.74a	20.64a	1.838a	1.897a
F-test	**	**	**	**	**	**	**	**	**	**
LxT	**	**	**	**	**	**	**	**	**	**

PL: Panicle length, PM: Panicle mass, SS; Seed set, HI: Harvest Index, SY: Seed yield

The results in Table (6) indicated that, the interaction between the CMS lines and foliar application were highly significantly affected on days to heading (DH), plant height (PH), number of fertile panicles hill^{-1} (NFPH), flag leaf angle (FLA), panicle exsertion (PE), panicle length (PL), panicle mass (PM), seed set (SS), harvest index (HI) and seed yield (SY) in both seasons. The CMS line IR58025A (L2) gave the highest values of all traits recorded under combination Cytokinin and Molybdenum, (111.74 and 110.52 day) of days to heading (DH), (120.93 and 122.77 cm) of plant height(PH), (20.76 and 22.46) of number of fertile panicles hill^{-1} (NFPH, (41.04 and 42.53 ^{0}) of flag leaf angle (FLA), (82.05 and 84.65%) of panicle exsertion (PE), (3.22 and 3.30 g) of panicle mass (PM), (29.00 and 31.60 %) of seed set (SS), (21.16 and 22.06 %) of harvest index (HI) and (1.916 and 1.975 t ha^{-1}) of seed yield (SY).While, The CMS line K17A (L5) recorded the lowest values of all traits under recorded without application (T1), days to heading (DH) (88.30 and 87.12 day), (81.45 and 83.29 cm) of plant height, (10.25 and 24.11) of number of fertile panicles hill^{-1} (NFPH), (24.11 and 25.21^{0}) of flag leaf angle (FLA), (42.89 and 45.50%) of panicle exsertion, (1.15 and 1.23 g) of panicle mass (PM), (7.75 and 10.35 %) of seed set (SS), (12.20 and 13.11 %) of harvest index (HI) and (0.638 and 0.697 t ha^{-1}) of seed yield (SY) during the both seasons.

Table 6. Effect of interaction between CMS liens and floralapplication on growth and yield component of CMS lines during 2021 and 2022 seasons.

Interaction		DTH (day)		PH (cm)		NFPH		FLA (%)		PE (%)	
		2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
L1	T1	105.32fg	104.12fg	87.33i	88.77j	10.53i	12.53i	24.35hi	25.85hi	52.34j	54.83j
(A1 ×	T2	106.36ef	105.16e	98.56f	100.41f	14.46e	16.16e	30.75d	32.25d	62.63e	65.23e
G178R)	T3	105.46fg	104.58fg	90.93h	92.77h	12.23h	13.93h	26.51g	28.01g	56.23gh	58.83gh
	T4	107.36de	106.16de	118.14b	119.98b	17.60bc	19.30bc	37.00b	38.48b	79.98b	82.59b
L2	T1	108.33cd	107.13cd	92.85gh	94.69gh	12.91gh	14.61efg	27.33fg	28.83fg	54.30i	56.93i
(A2 ×	T2	109.76bc	108.56bc	111.00c	112.84c	18.40b	20.10b	35.54b	37.04b	66.05d	68.64d
G178R)	T3	110.70ab	109.56ab	107.38d	109.23d	15.86d	17.56d	30.56d	32.08d	57.30g	59.90g
	T4	111.74a	110.52a	120.93a	122.77a	20.76a	22.46a	41.04a	42.53a	82.05a	84.65a
L3	T1	104.24g	103.05h	95.19g	97.03g	11.16i	12.86i	26.38g	27.88g	56.04gh	58.64ghi
(A3 ×	T2	106.28ef	105.08ef	110.00c	111.84c	16.71cd	17.50d	33.67c	35.16c	65.34d	67.93d
G178R)	T3	104.52g	103.32gh	101.30e	103.14e	14.00ef	15.70ef	32.59c	34.98c	60.55f	63.15f
	T4	106.66ef	105.46ef	120.85a	122.77a	17.08c	18.78c	36.60b	38.16b	79.76b	82.36b
L4	T1	88.35j	87.13k	92.74gh	94.58gh	10.57i	12.27i	29.12de	30.62de	51.18j	53.78j
(A4 ×	T2	91.79h	90.59i	110.30c	112.17c	16.71cd	18.41cd	33.30c	34.83c	70.67c	73.26c
G178R)	T3	90.79hi	89.59ij	101.03ef	102.87e	13.42efg	15.12efg	32.48c	33.98c	60.90ef	63.50ef
	T4	90.42hi	89.22ij	122.40a	124.24a	18.33b	20.03b	35.36b	36.86b	79.22b	81.52b
L5	T1	88.30j	87.12k	81.45j	83.29j	10.25i	11.94i	24.11i	25.21i	42.89k	45.50k
(A5 ×	T2	89.39ij	88.19jk	110.43c	112.27c	16.60cd	18.30cd	28.29ef	29.89ef	62.67e	65.27e
G178R)	T3	88.35j	87.15k	92.22h	94.06h	13.10fgh	14.80fgh	26.00gh	27.50gh	55.33i	57.93hi
	T4	90.39hi	89.19ij	116.00b	117.84b	17.33bc	19.03bc	32.72c	34.22c	79.68b	82.28b

DTH: Days to heading, PH: Plant height, NFPH: Number of fertile panicles hill-1, FLA: Flag leaf angle, PE: Panicle exsertion

Contented Table.6

Interaction		PM (g)		SS (%)		HI (%)		SY (t ha^{-1})	
		2021	2022	2021	2022	2021	2022	2021	2022
L1 (A1 × G178R)	T1	1.19p	1.08q	8.90j	11.50j	13.31kl	14.21kl	0.743n	0.802n
	T2	2.39g	1.37n	16.72gh	19.32gh	17.55f-i	18.45f-i	1.493i	1.552i
	T3	1.29n	2.47g	22.21e	24.81e	19.35bcd	20.25bcd	1.740e	1.799e
	T4	2.69d	2.77d	25.00c	27.60c	20.33ab	21.24ab	1.906ab	1.965ab
L2 (A2 × G178R)	T1	1.39m	1.47m	11.51i	14.11i	14.29k	15.19k	0.872m	0.931m
	T2	2.63e	2.71e	18.32f	20.92f	15.66j	16.56j	1.715f	1.775f
	T3	2.99c	3.07c	23.44d	26.04d	19.01cde	19.91cde	1.801c	1.860c
	T4	3.22a	3.30a	29.00a	31.60a	21.16a	22.06a	1.916a	1.975a
L3 (A3 × G178R)	T1	1.27no	1.35no	8.42jk	11.02jk	12.52lm	13.43lm	0.710o	0.769o
	T2	2.27i	2.35i	17.24g	19.83g	17.24ghi	18.14ghi	1.477j	1.536j
	T3	2.59f	2.67f	23.21d	25.58e	19.08cde	19.98cde	1.713f	1.773f
	T4	3.12b	3.20b	28.00b	30.60b	20.20abc	21.10abc	1.897b	1.956b
L4 (A4 × G178R)	T1	1.25o	1.33o	8.07jk	10.67jk	12.09m	12.99m	0.672p	0.732p
	T2	2.20k	2.28k	16.29h	18.89h	17.01hi	17.91hi	1.442k	1.501k
	T3	2.29i	2.37i	21.98e	24.58e	17.47f-i	18.37f-i	1.653g	1.712g
	T4	3.01c	3.09c	27.33b	29.93b	18.66def	19.56def	1.710f	1.769f
L5 (A5 × G178R)	T1	1.15q	1.23p	7.75k	10.35k	12.21lm	13.11lm	0.638q	0.697q
	T2	2.16l	2.24l	18.36f	20.96f	16.41ij	17.31ij	1.392l	1.451l
	T3	2.24j	2.32j	24.60c	27.20c	18.02e-h	18.92e-h	1.602h	1.660h
	T4	2.32h	2.40h	28.00b	30.60b	18.33d-g	19.23d-g	1.740e	1.819d

PM: Panicle mass, SS; Seed set, HI: Harvest Index, SY: Seed yield

DISCUSSION

The effects of foliar spraying treatments of Cytokinin (Ck) and Molybdenum (Mo) improved floral traits of male parent were due to increase activity of cell division, enlargement, and elongation. Combination between Cytokininand Molybdenum are plant hormones that regulate various processes of plant growth and development, which are particularly important in cell elongation The improved floral traits of male parent were due to increased activity of cell division, enlargement and elongation. Combination between cytokines and mol bedim are plant hormones that regulate various processes of plant growth and development, which are particularly important in cell elongation (Hedden and Phillips, 2000). The results agree with those reported by Rahman *et al.* (2010) and Abo-Youssef *et al.* (2017). The variation between the CMS lines could be attributed to the difference in genetic background. The linesIR70368A(L3) and K17A (L5) were the best lines among studied Lines for most studied floral traits. The results agree with those reported by (Hamad *et al.,* 2021). They found that, the different doses of GA3 showed highly significantly influence on floral traits. Similar results agreements with those were reported by Ehsan and Robert (2019). The results for the interaction between the lines and treatments showed highly significant effects for treatments lines – interaction, the results are in agreement with those reported by Sirajul *et al.* (2005); Gavino *et al.* (2008). Riaz *et al.* (2019); Ghoneim (2020). The fourth treatment (T4) which has a mixture of Ck and Mo gave the best results for all studied growth characters on all studied genotypes due to the complementary of both components. Similar results agreements with those were reported by Ehsan and Robert (2019). The lines IR58025A (L2) and R70368A(L3) were the best lines among studied CMS lines for most studied growth and yield traits. The results in **Table (6)** indicated that the interaction between the CMS lines and foliar application were highly significantly affected onall studied growth and yield traits. T4 increased the hybrid seed yield by around one ton /hectare comparing with controlby increasing the seed set percentage and improving out cross rate for both parental lines.The results are in agreement with those reported by Abo-Youssef (2009), Hao *et al.* (2010) and Faiz *et al.* (2017).

CONCLUSION

Due to the importance of Growth regulators including the Cytokinins (CKs) and the importance of the Molybdenum (MO) as a cofactor to promote some enzymes and nitrogen fixing in plant. Using the mixture of (CKs) and (MO)with ratio 1:1 as a foliar application two times before heading improved the Parental lines performance for out crossing rate, increased the seed set percentage and total seed yield in hybrid rice seed production.

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تحسين أداء السلالات الأبوية بإضافة السيتوكينين والموليبدينوم على إنتاج تقاوى الأرز الهجين

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أجريت تجربة حقلية بمزرعة محطة البحوث الزراعية بسخا كفرالشيخ مصر خلال موسمى 2021 و 2022 و كان الهدف من التجربة هو دراسة تأثير السيتوكينين والموليبدينوم علي إنتاجية تقاوى الأرزالهجين . تضمنت التجربة أربعة معاملات (T1 – بدون إضافة ، T2 – سيتوكينين بتركيز 20 جزء في المليون ،T3 – موليبدنوم بتركيز 35 جزء في المليون و :1 - T4 1خليط من السيتوكينين 10 جزء في المليون والموليبدينوم 17.5 جزء في المليون) للاب الملقح جيزة. 178 والسلالات العقيمة ذكر ياسيتوبلازميا (IR69625 وIR70368 وIR58025 و K17وG46) على إنتاجية تقاوى الأرز الهجين .كان التصميم المستخدم فى هذه التجربة هو القطع المنشقة مرة واحدة في ثلاث مكررات. إضافة السيتوكينين والموليبدينوم أدىإ لتحسين معدل التهجين للاب الملقح من خلال التأثير على الصفات الزهرية وبالتالي زيادة إنتاج تقاوى الأرز الهجين. التأثير الأكثر معنوية للمعاملات المطبقة كانت على طول المتك وعرض المتك و خصوبة حبوب اللقاح وعدد حبوب اللقاح وعرض الريشة وطول الريشة وعدد الأيام حتى التزهير وعدد الفروع الحاملة للداليات الخصبة فى الكن و ارتفاع النبات وطول الدالية ونسبة العقد ومحصول الحبوب. للاب الملقح جيزة R178 مع المعاملة T4 السيتوكينين و الموليبدينوم) .زاوية تفتح السنيبلة ،مدة تفتح السنيبلة ،إجمالي طول الريشة ،طول الخيط ،طول الريشة ،عرض الريشة ،بالإضافة إلى ذلك ،أظهرت السلالات العقيمة ذكريا سيتوبلازميا هذه أعلى نمو للنبات ومحصول التقاوى ،خاصة مع المعاملة T4. وبالتالي ،يمكن اضافة لـ (T4) ، وهى السيتوكينين مع الموليبدينوم لتحسين الصفات الزهرية والنمو والإنتاجية للسلالات العقيمة ذكريا سيتوبلازميا المبشرة مثل السلالة 1 والسلالة 3 والسلالة4 ،وبالتالي زيادة معدل التهجين وإنتاج تقاوى الارزالهجين.

الكلمات المفتاحية: انتاج الأرز الهجين ، تحسين التلقيح الخلطي، السيتوكينين، الموليبدينوم.