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Studies on Japonica Hybrid Rice Seed Production under Egyptian Conditions

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Abstract

Two- experiments were conducted at Sakha Experimental Farm of Sakha Agricultural Research station, Kaferelsheikh, Egypt during 2016 and 2017 rice growing seasons. The objective of this investigation was to study the effect of three Male(P):Female(S) rows ratio (2P:6S, 2P:8S and 2P:10S) and two pollen directions of the pollen parental line (P), one direction and two directions on out-crossing rate for Japonica hybrid rice seed production. Japonica hybrid produce through crossed between one Thermo-sensitive Genic Male Sterile (TGMS) line (TG-60-6) as a female line (S) with Sakha105 which used as a male pollinator line (P). A split-plot design with three replications was used. The main plots were dedicated to rows ratio whereas direction of the pollen parental line was arranged within the sub plots. The results notify that the better row ratio was 2P:6S gave the best seed yield hill-1 (25.00 and 27.38 g) in 2016 and 2017 seasons respectively. For direction of the pollen parental line, the best F_1 seed yield hill^{-1} (25.89 and 27.45 g) were obtained once planted the spore parental line in two directions during 2016 and 2017 seasons, respectively. The interaction between row ratio and direction of the pollen parental line was highly significant for panicle weight (g), seed set (%), number of fertile panicles hill-1, harvest index (%), and seed yield hill-1, but not significant for plant height (cm) and panicle length (cm) during 2016 and 2017 seasons. The results notify the importance of optimum row ratio and direction planted of pollen parental line for increasing the out-crossing rate in Japonica hybrid seed

Key words: *Japonica hybrid rice (Oryza sativa L.), TGMS, Row ratio, Direction of pollen parental line, seed yield.*

Introduction

Hybrid rice is the commercial rice seed which produced from a cross between two genetically dissimilar parental lines and grow as F_1 seed. Three-line hybrids have the potential of yielding 15–20% more than the best inbred variety grown under similar conditions **(Virmani et al, 1997)**. In 1995, Yuan Long Ping declared utilization of two-line hybrid rice has basically succeeded since then many new two line hybrid combinations have been developed, released and used on large scale commercial production in china. Two-line hybrid rice generally has 5-10 % yield advantage over the three-line hybrid rice combinations **(Jiming and Long Ping, 2007)**. Produce of Japonica hybrid rice depend on Environmental Genic Male Sterility (EGMS) phenomenon. The EGMS is composed of two major types; Photo-sensitive Genic Male Sterility (PGMS) which is responsive to variations in day length, and Thermo-Sensitive Genic Male Sterility (TGMS) which is caused by temperature. A Fertility/Sterility Alternation of TGMS line is induced mainly by temperature. The existing TGMS lines become completely male sterile under higher temperature. TGMS trait is governed by major genes, thus enabling their easy transfer to any genetic background, no need for restorer genes in the male parents of two-line hybrids, this system is ideal for developing Japonica hybrids because most Japonica lines doesn't possess restorer genes **(Mou-Tong *et al*., 2003)**.

The discovery of Environmental Genic Male Sterility (EGMS) was help rice breeder to produce of simple two-line Japonica hybrid rice. Compared with the three-line system, the two-line system (EGMS) has many advantages such as no need to a maintainer line for seed multiplication, thus making seed production simpler and more cost-effective. Maintaining grain self-sufficiency in Egypt has become hard mission for most reasons such as the population grows, water shortage and arable land shrinks, so the pressure on Egyptian food security is severe increasing. In general, there are two ways to increase the grain yield output of rice; expanding planting areas and increasing grain yield per unit area. However, expanding the planting area for rice in Egypt is not available due to water restrictions and strong competition from other crops. Therefore, the only one way to increase rice production is increasing rice grain yield. We can increase rice grain yield from unit area through produce high yield Japonica hybrids coupled with good grain quality **(Zaman *et al*., 2002)** that is minor goal from this investigation, and then increase the income of Egyptian farmers and the improvement of the Egyptian economy, which is the major goal from this study.

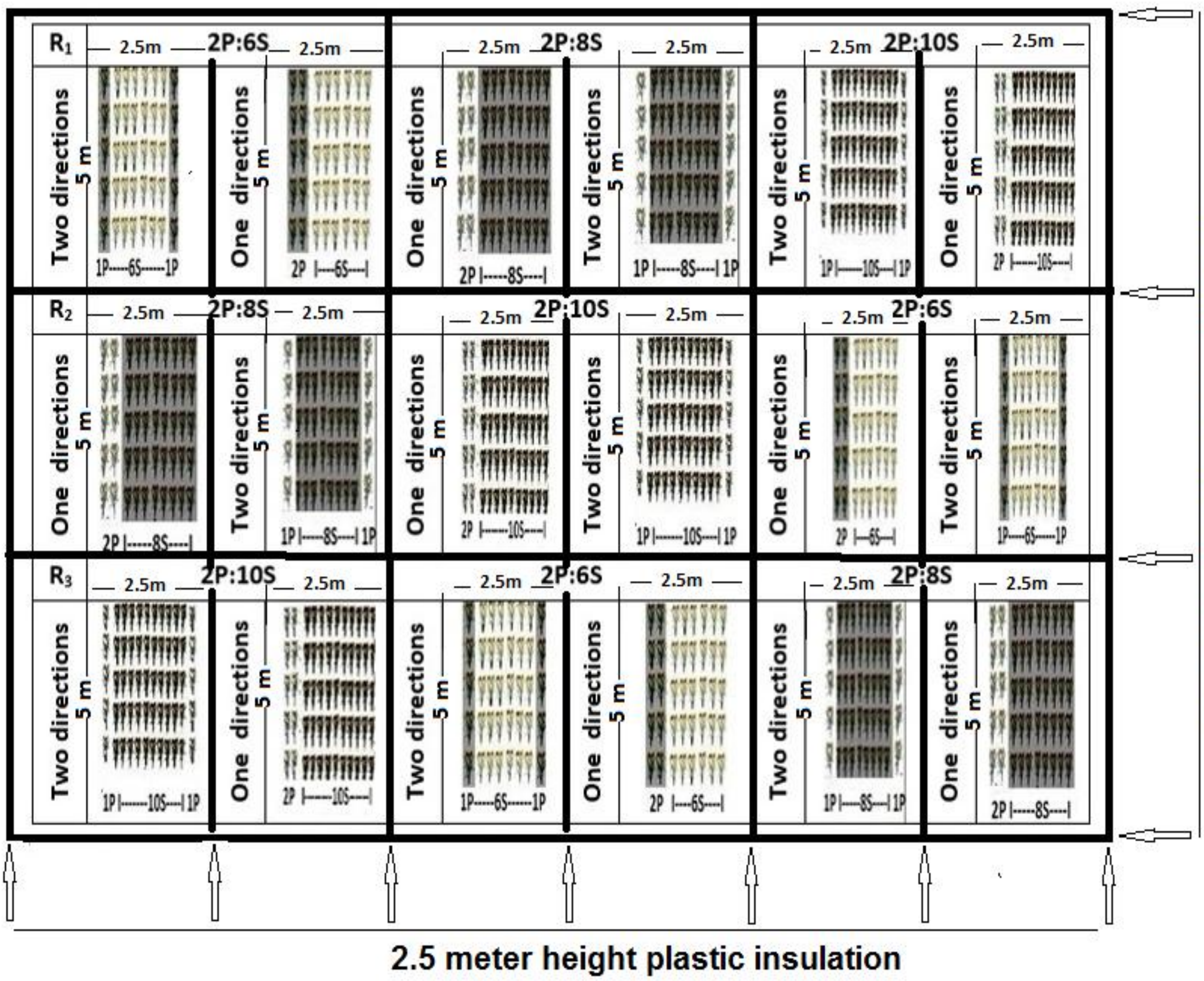
Material and Methods

The experimental materials were include one Thermo-sensitive Genic Male Sterile (TGMS) line (TG-60-6) developed in Egypt which was used as female line (S) and Sakha105 as pollen parent (P). The female TGMS line TG-60-6 was produced from hybridization between one reverse Thermo-sensitive Genic Male Sterile Line M.J5460s (china) with Sakha106 (Egypt) followed by pedigree selection methods in segregation generation from F_2 to F_6 into stability. This line was evaluated under different sowing dates to select best sowing date to induce complete sterility which used to produce Japonica hybrid rice in seed production program. Experimental materials, parentage origin and grain type were shown in **Table 1**.

Table 1. Rice parental lines, parentage origin and grain type

No.	Entries	Parentage	Origin	Grain type
1	TG-60-6	(M.J5460 S x Sakha106)	Egypt	Japonica
2	Sakha105	(GZ 5581 x GZ 4316)	Egypt	Japonica

A split-plot design in RCBD arrangements with three replications was used. The main plots were devoted to the pollen parental line (P) to the TGMS line (S) row ratio (2P:6S, 2P:8S and 2P:10S) and direction of pollen parental line (one direction and two direction) were arranged in the sub plots with size 12.5 m^2 (2.5 x 5m) for each sub plot. The experiment border and each sub plot were isolated by a 2.5 meter height plastic insulation such are shown in **figure 1**.



Spacing: between pollen parental line `P' rows: 30 cm**,** between TGMS line `S' rows: 15 cm, between `P' & `S' line blocks: 20–30 cm and between hills (`S' & `P' lines): 15 cm

Fig. 1 Design and arrangement of experiment, P-S row ratio and P transplanted directions.

Thermo-sensitive Genic Male Sterile (TGMS) line (TG-60-6) sown on May 3rd and pollen parent Sakha 105 was sown at three different sowing date (April 22th, April 25th and April 28th) to get a proper synchronization of flowering depend on days from planted to start flowering for both parental female and male lines. (TGMS) line (TG-60-6) earlier six days than pollen parent Sakha 105 which were 90 and 96 days for TG-60-6 and Sakha105, respectively. TG-60-6 was start flowering at August 2nd and continuous receipt pollen during 7th days. While, every sowing date form Sakha105 as pollen parental line supply the female line with pollen grain during 5 days as shown in **Figure 2**.

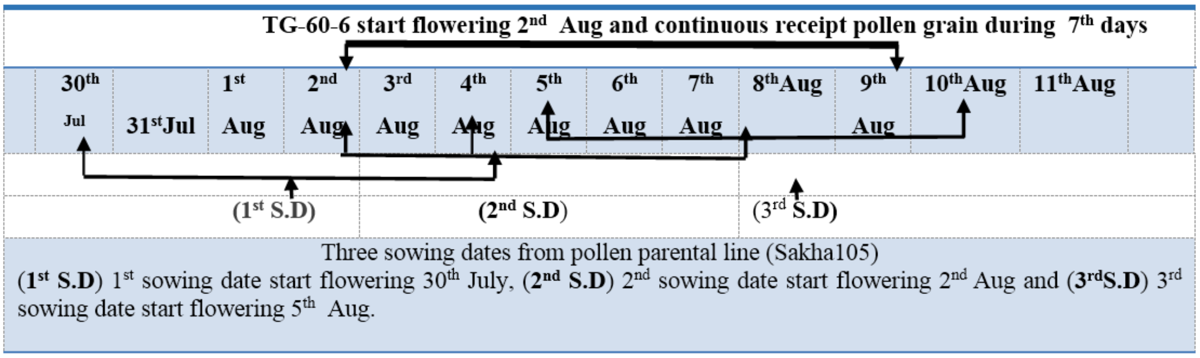


Fig. 2 flowering synchronization depends on days to flowering for parental lines TG-60-6 and Sakha105.

Days old seedlings of both P and S lines were transplanted by 3-4 and 2 seedlings per hill, respectively. The row spacing maintained for P-P, P-S, and S-S lines were 20, 30, and 15 cm, respectively. Hill spacing for both P and S lines were maintained at 15 cm. Experiment grown under Isolation for hybrid seed production. Rice seeds at the seeding rate of 20 kg/ha (15 kg from TGMS line (TG-60-6) and 5 Kg from the parental line Sakha105 were soaked in a fresh water for 24 hours, then drained and incubated for 48 hours to hasten early germination.

The nursery seedbed was well ploughed and dry leveled. Phosphorous fertilizer was added in the form of single super phosphate (15.5% P_2O_5) at the rate of 240 kg/ha before tillage. Nitrogen in the form of urea (46% N) at the rate of 145 kg/ha was added at the rate of (95 kg /ha as basal dressing, and the second 50 kg/ha at panicle initiation. Zinc sulphate 22 % $ZnSO_4$ at the rate of 50 kg /ha was added after puddling and before planting. Data were collected for; plant height (cm), panicle length (cm), No of fertile panicles/hill, panicle weight (g), seed set (%), harvest index (%) and seed yield hill^{-1}(g). The crop was harvested when 80 % of the grains became golden yellow in color. Grains were sun dried and adjusted at 14% moisture content to estimate grain yield.

Harvest index (HI %) percentage was estimated according to the following formula:

$$\text{Harvest index (HI \%)} = \frac{\text{Grain yield (economic yield)}}{\text{Biological yield}} \times 100$$

Seed set percentage was calculated according to the following formula

$$\text{Seed set \%} = \frac{\text{Number of filled grains panicle}^{-1}}{\text{Total spiklet number panicle}^{-1}} \times 100$$

The data were collected according to Standard Evaluation System of **IRRI (2013)** for all the studied characters. All cultural practices were practiced as recommended for hybrid rice seed production package. The data were analyzed following the ANOVA analysis and the mean differences were compared by the Duncan's Multiple Range Test **Duncan (1956).** Using a statistical computer package COSTAT **(Gomez and Gomez, 1984)**.

Data of climate and weather conditions at the Farm of Sakha Agricultural Research Station, ARC, Egypt were recorded during two growing seasons 2016 and 2017 from 10th May to 30th Sep., Meteorological data regarding every ten days and the monthly means of wind velocity km/24 hr., relative humidity (R.H. %) and air temperature (°c) are shown in Table 2. Especially, air temperature data must be considered because it is important factor to induce sterility of female line (TG-60-6) as Thermo-sensitive Genic Male Sterility (TGMS). TGMS lines were complete sterility in air temperature more than 32 °c as maximum air temperature. Minimum of four continuous weeks with stable high temperature (>32/24°c day/night) for safe hybrid seed production.

Microscopic analysis for the TG-60-6 Line as female line and pollen parental line Sakha105. Pollen examination was made on five randomly plants from each replication to insure sterility in TG-60-6 and Sakha 105. Five spikelet's were collected from each main panicle and fixed in 70 % alcohol. From these spikelet's 6 anthers were collected and smeared in iodine potassium iodide (IKI) solution (1%) and examined under light microscope (40x10). All the unstained pollens were considered as sterile and the stained ones as fertile **(Virmani *et al*, 1997).** Bagging of five TG-60-6 plants from each sub plot by rate thirty plants from each replication and estimated seed set in these plants to judge in sterility of TG-60-6 line.

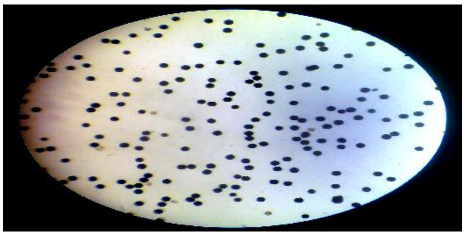
Table 2 Climate and weather data during two growing seasons 2016 and 2017

Date	Wind Velocity km/24 hr		RH%				Air Temperature			
			1.30 PM		7.30 AM		Min		Max	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
10 May	105.2	94.6	47.5	46.9	80.3	76	17.27	20.98	29.15	28.8
20 May	124.5	96.2	44.6	41.5	79.2	65.6	18.38	24.36	30.32	32.53
30 May	114.00	100.1	46.30	48.90	72.50	71.4	20.71	22.93	31.10	29.72
Mean	114.60	97.00	46.10	45.80	77.30	71.00	18.79	22.81	30.19	30.40
10 June	99.30	91.20	50.60	43.50	79.10	67.30	20.19	26.70	30.03	33.40
20 June	103.90	130.40	52.30	44.30	80.20	76.00	22.05	25.50	31.45	33.10
30 June	113.30	116.80	50.70	52.10	77.00	83.90	21.98	26.80	31.09	34.20
Mean	105.30	112.80	51.20	46.60	78.80	75.70	21.40	26.30	30.85	33.60
10 July	114.10	109.90	53.50	57.50	82.80	83.40	24.80	26.50	32.80	33.90
20 July	90.10	99.10	57.60	54.20	88.40	83.00	25.30	25.90	33.40	33.70
30 July	87.60	107.40	51.80	58.60	87.40	81.70	26.30	26.00	34.30	32.90
Mean	97.30	105.50	54.30	56.80	85.20	82.70	25.46	26.13	33.00	33.70
10 Aug.	84.60	87.90	52.50	57.60	83.50	84.80	26.20	26.20	35.90	34.30
20 Aug.	93.20	93.00	51.60	56.80	81.60	83.10	25.50	26.30	35.50	33.00
30 Aug.	95.90	97.60	51.10	54.40	86.40	85.00	24.30	25.50	33.90	33.90
Mean	91.20	92.80	51.70	56.30	83.80	84.30	25.00	26.00	35.10	33.60
10 Sep.	84.60	86.80	52.50	53.70	83.50	85.40	26.20	24.30	35.90	33.27
20 Sep.	93.20	94.20	51.60	51.30	81.60	82.60	25.50	24.93	35.50	33.42
30 Sep.	95.90	104.30	51.10	50.30	86.40	81.40	23.30	23.63	33.90	31.09
Mean	91.20	95.10	51.70	51.80	83.80	83.10	25.00	24.30	35.10	32.60

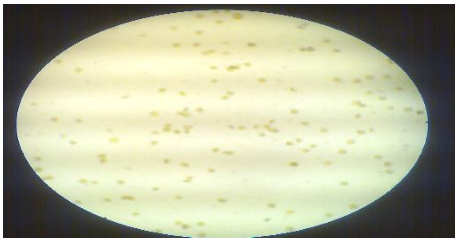
Results and Discussion

Above all, it is necessary to clarify important of spikelet's bagging and pollen examination to safe hybrid rice seed production. To be sure the seed set in the female line were hybrid seed and not resulted from self-pollination. Bagging of plants indicated that, no seed set % at bagging female line TG-60-6. Pollen examination revealed that, complete sterility for TG-60-6 as female line comparing with complete fertility for Sakha105 as pollen parent line as shown in figure3.

Effect of pollen parent Sakha 105 of the female TGMS rows ratio and direction of the male parent as well as their interaction on plant height, panicle length, panicle weight and number of fertile panicles hill^{-1} characters are given in **(Table 3)**.



(a) Male fertility (Sakha105)



(b) Male sterility (TG-60-6)

Fig. 3 Show (a) male fertility pollen parental line (Sakha105), (b) male sterility female line (TG-60-6) as thermo-sensitive genic male sterility line for pollen examination.

Pollen parental line to the TGMS row ratio effect

The effect of the pollen parental line to TGMS row ratio was not significant for plant height and panicle length during 2016 and 2017 seasons. While, gave highly significant effect for panicle weight and number of fertile panicle hill^{-1}, the parental line to TGMS ratio 2P:6S produced the heaviest panicles (1.82 and 2.02 g) and (16.54 and 17.03 /hill) for fertile panicles hill^{-1} during both seasons, respectively. By contrast, the male parent Sakha 105 to TGMS row ratio 2P:10S produced the lowest panicles (1.58 and 1.67 g) and (14.97 and 14.90) for fertile panicles hill^{-1} in 2016 and 2017 seasons, respectively. **Rahman *et al* (2010)** studied the effect of row ratio of restorer (R) and Cytoplasmic Male Sterility (A) lines on seed production of hybrid rice. Similar results were also found by **Singh and Singh (1998).**

Direction of the pollen parental line effect

Direction of the pollen parent Sakha 105 **was** not significant for the characters, plant height and panicle length. While, was highly significant effect for panicle weight and fertile panicles hill^{-1} as shown in **Table 3**. Two direction parent Sakha 105 produced the heaviest panicles (1.93 and 2.05 g) and (16.09 and 16.87) for fertile panicles hill^{-1} during 2016 and 2017 seasons, respectively. On the other hand, one rows direction of pollen parent Sakha 105 produced the least panicle weight (1.84 and 1.60 g) and gave (15.29 and 14.95 hill^{-1}) for fertile panicles hill^{-1} in 2016 and 2017 seasons, respectively. The results are in agreement with those reported by **Huang *et al* (1998) and Zhu *et al* (1998).**

Interaction effect

The interaction between pollen parent Sakha 105 for TGMS rows ratio and direction of pollen parent Sakha 105 has highly significant for panicle weight and fertile panicles hill^{-1} in 2016 and 2017 seasons, respectively. While, was not significant for the characters of plant height and panicle length in both seasons.

Table 3. Effect of pollen parental line to TGMS row ratio and direction of the pollen parental line, as well as, their interaction on plant characteristics during 2016 and 2017 seasons

Main effect and interaction	Plant height (cm)		Panicle length (cm)		Panicle weight (g)		Number of panicles/hill	
	2016	2017	2016	2017	2016	2017	2016	2017
Row ratio								
2P:6S	80.92	84.64	15.02	101.8	1.82a	2.02a	16.54a	17.03a
2P:8S	80.87	84.42	14.76	98.12	1.73b	1.78b	15.56b	15.80b
2P:10S	80.86	84.66	14.58	94.28	1.58c	1.67c	14.97c	14.90c
F-Test	NS	NS	NS	NS	**	**	**	**
Direction								
One Direction	80.78	84.61	14.94	14.65	1.84b	1.60b	15.29b	14.95b
Two Direction	80.99	84.53	14.64	14.62	1.93a	2.05a	16.09a	16.87a
F-Test	NS	NS	NS	NS	**	**	**	**
Interaction (R x D)	NS	NS	NS	NS	**	**	**	**

** Highly significant at the 1% level of probability, NS not significant

The results in Table 4 showed that the interaction between pollen parent Sakha 105 and TGMS rows ratio and direction of pollen parent Sakha 105 has highly significant effect on panicle weight and fertile panicles hill^{-1} in both seasons 2016 and 2017, respectively. The pollen parent with TGMS ratio 2P:6S and two direction of pollen parental Sakha 105 gave the highest values of 2.15 and 2.22 g for panicle weight and gave 17.90 and 17.80 for fertile panicles hill^{-1} in 2016 and 2017 seasons, respectively. While, male parent Sakha 105 with TGMS rows ratio 2P:10S with one direction parent lines produced the lowest values of 1.40 and 1.47 g for panicle weight and (14.87 and 14.00/hill) for number of fertile panicles/hill during both seasons, respectively. Similar results were also found by **Prabagaran and Ponnuswamy (1997) and Kumar and Nautiyal (2016).**

Table 4. Effect of interaction between parental line to TGMS ratio and direction parent lines on panicle characteristics during 2016 and 2017 seasons

Direction	Row ratio	Panicle weight (g)		Number of panicles /hill	
		2016	2017	2016	2017
One Direction	2P:6S	1.57d 1.49	1.82c	15.90c	16.25c
	2P:8S	e	1.52e	15.1d	14.60d
	2P:10S	1.40 f	1.47f	14.87f	14.00f
Two Direction	2P:6S	2.15a	2.22a	17.90a	17.80a
	2P:8S	1.89b	2.10b	16.02b	17.01b
	2P:10S	1.77c	1.82d	15.07e	15.80e

Pollen Parent Sakha 105 with TGMS line rows ratio effect

The effect of Parent Sakha 105 to TGMS ratio has highly significant effect on the characters of seed set percentage, harvest index and seed yield were showed in table 5. The pollen parental line to TGMS rows ratio 2P:6S gave the highest values of 27.85 and 28.67 % for seed set, (16.58 and 17.58 %) for harvest index and (25.89 and 27.45 g/plant) for seed yield in during 2016 and 2017 seasons, respectively. But, the Parental line to TGMS row ratio 2P:10S produced the lowest values (23.86 and 24.68 %) for seed set, (14.08 and 14.53 %) for harvest index and (19.92 and 21.32 g) for seed yield plant^{-1} in the first and second seasons, respectively. The results are in agreement with those reported by **Abo-Youssef, (2009), Viraktamath and Ramesha (1996).**

Direction of pollen parent Sakha 105 effect

Direction of male parental line has highly significant effect on seed set, harvest index and seed yield. Two directions produced the highest values (28.77 and 29.22 %) for seed set, (16.55 and 17.93%) for harvest index and (25.89 and 27.45 g) for seed yield hill^{-1} in 2016 and 2017 seasons, respectively. By contrast, one direction gave the lowest values (23.04 and 24.27 %) for seed set, (13.83 and 14.17 %) for harvest index and (18.83 and 21.06 g) for seed yield plant^{-1} in 2016 and 2017 seasons, respectively. Similar results were also found by **El-Mowafi *et al* (2016) and Virmani (2003).**

Table 5. Effect of Parental line to TGMS ratio and direction parental line, as well as, their interaction on seed set, harvest index and seed yield during 2016 and 2017 seasons

Main effect and interaction	Seed set (%)		Harvest index (%)		Seed yield hill^{-1} (g)	
	2016	2017	2016	2017	2016	2017
Row ratio						
2P:6S	27.85a	28.67a	16.58a	17.58a	25.00a	27.38a
2P:8S	26.03b	26.89b	14.91b	16.04b	22.16b	24.07b
2P:10S	23.86c	24.68c	14.08c	14.53c	19.92c	21.32c
F-Test	**	**	**	**	**	**
Direction						
One Direction	23.04b	24.27b	13.83b	14.17b	18.83b	21.06b
Two Direction	28.77a	29.22a	16.55a	17.93a	25.89a	27.45a
F-Test	**	**	**	**	**	**
Interaction (R x D)	**	**	**	**	**	**

** Highly significant at the 1% level of probability.

Interaction effect

The interaction between pollen parent Sakha 105 to TGMS rows ratio and direction of the parental line was highly significant for seed set, harvest index and seed yield during 2016 and 2017 seasons, respectively. The results in Table 6 showed that the interaction between pollen parental line to the TGMS row ratio and direction of pollen parental line has highly significant effect on seed set, harvest index and seed yield in both seasons. The pollen parent line to TGMS row ratio 2P:6S with two direction pollen parental line gave the highest values (30.50 and 31.04 %) for seed set %, (18.26 and 20.03 %) for harvest index and (29.47 and 30.16 g/plant) for seed yield in 2016 and 2017 seasons, respectively. While, pollen parent to TGMS rows ratio 2P:10S with one direction pollen parent produced the lowest values (20.17 and 22.02 %) for seed set, (13.05 and 13.22 %) for harvest index and (16.77 and 17.54 g/plant) for seed yield during both seasons, respectively. Similar results were obtained by **Jia *et al* (2001).**

Table 6. Effect of interaction between pollen parental line to TGMS row ratio and direction of pollen parent lines on seed set, harvest index and seed yield during 2016 and 2017 seasons

Direction	Row ratio	Seed set (%)		Harvest index (%)		Seed yield hill^{-1} (g)	
		2016	2017	2016	2017	2016	2017
One Direction	2P:6S	25.20d	26.30d	14.90d	15.13d	20.53d	24.60d
	2P:8S	23.19e	24.5e	13.57e	14.17e	19.17e	21.06e
	2P:10S	20.17f	22.02f	13.02f	13.22f	16.77f	17.54f
Two Direction	2P:6S	30.5a	31.04a	18.26a	20.03a	29.47a	30.16a
	2P:8S	28.81b	29.30b	16.26b	17.91b	25.15b	27.09b
	2P:10S	27.02c	27.33c	15.14c	15.84c	23.07c	25.08c

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دراسات على انتاج تقاوى الأرز الهجين اليابانى تحت الظروف المصرية

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أجريت تجربة فى موسمي 2016 و2017 بمزرعة محطة البحوث الزراعية بسخا– كفر الشيخ بهدف دراسة تأثير نسب سطور الأب للأم (2 أب:6 أم و2 أب:8 أم و2 أب:10 أم) مع إتجاه شتل الأب (فى اتجاة واحد وفى اتجاهين) علي إنتاجية تقاوى الأرز الهجين اليابانى ،الناتج من السلالة الحساسة لدرجة الحرارة كسلالة عقيمة مع الصنف اليابانى سخا105 كأب ملقح . كان التصميم المستخدم هو قطع منشقة مرة واحدة في ثلاث مكرات حيث تم وضع نسب السطور للأب الخصب الى الأم ذات العقم الذكرى الوراثى البيئي المتأثرة بإرتفاع درجة الحرارة في القطع الرئيسية ووضعت معاملات اتجاهات الأب بالنسبة للأم في القطع المنشقة. أوضحت النتائج أن إرتفاع النبات وطول الدالية كانت غيرمعنوية مع تأثير نسب سطور الأب الخصب الى الأم العقيمة وإتجاهات شتل سطور الأب، وعلى الجانب الأخر تأثرت عدد السنابل الخصبة فى الجورة ووزن السنبلة ونسبة العقد و دليل الحصاد و محصول التقاوى معنويا بتغير نسب الأب الخصب الى الأم العقيمة 2 أب:6 أم و إتجاه شتل الأب فى إتجاهين. وكان محصول الحبوب29.47 و 30.16 جرام للنبات عند إستخدام النسب 2 أب مع 6 سطور أم و إتجاه شتل الأب فى إتجاهين خلال موسمى 2016 و2017. النتائج السابقة أعطت أهمية نسب الأب للأم المثلى وإتجاه شتل الأب لزيادة معدل التلقيح الخلطى فى حقل إنتاج تقاوى الأرز الهجين اليابانى.