



Proportion of parental line (A receptor and R pollinator) seeds improving rice hybrid production

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ABSTRACT. The high price of rice hybrid seeds is one of the major limiting factors for increasing the use of this technology. An important step toward minimizing high rice hybrid seed cost is developing techniques that help improve hybrid seed yield. Our goal was to determine the proportion of seeds of line A (receptor) and line R (pollinator) that, under mixed sowing, would allow optimized rice hybrid seed production and grain quality. Trials were performed for two growing seasons in the southern region of Brazil. The cultivar INTA Puita was used as a pollinator and line 464 A as receptor. The experimental design was a randomized block with four replications. Treatments included six proportions of seeds of the INTA Puita (0.75, 1.5, 3.0, 6.0, 12.0, and 24.00%) mixture with seeds of line 464 A (99.25, 98.50, 97.00, 94.00, 88.00, and 76%, respectively). By increasing the percentage of the pollinator seeds (INTA Puita CL) from 0.75 to 24% in the mixture with the receptor (line 464 A), we increased yield of INTA Puita CL grains (7 to 2676 kg ha⁻¹) and hybrid seeds (279 to 1493 kg ha⁻¹). The mixture of different proportions of INTA Puita CL (pollinator) with the receptor (line 464 A) did not change the rice grain quality or the production cost. The cost of production per kg of hybrid seeds decreased quadratically with the increasing percentage of the pollinator (INTA Puita CL), from US\$ 6.71 to US\$ 1.23. Revenue achieved from the commercialization of the grains of INTA Puita CL with hybrid seeds 464 A showed quadratic increases with the increased percentage of INTA Puita cl in the mixture. Our results showed a potential technique that could be used to improve hybrid seed production, reduce cost per kg of hybrid seed and improve revenue from producing rice hybrid seeds.

Keywords: *Oryza sativa*; agronomic strategies; pollinator rates; male sterile.

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Introduction

Rice is a staple food for more than half of the world's population (Prasad, 2011; Nascente, Crusciol, & Cobucci, 2013; Tesio, Tabacchi, Cerioli, & Follis, 2014). In addition, demand for this grain will increase worldwide; it is expected that approximately 116 million additional metric tons will be required in the year 2035 compared with the world rice production in 2010 (GRiSP, 2013). Therefore, it is necessary to develop technologies that allow increasing rice grain yield in order to meet this demand (Akhter, Sabar, Zahid, & Ahamd, 2007; Qin et al., 2013). Hybrid rice is a technology that allows increased grain yield by 15-30% (Krishnakumar, Nagarajan, Natarajan, Jawahar, & Pandian, 2005; Ravi, Ramesh, & Chandrasekaran, 2007; Virmani & Kumar, 2014), which represents an increase in productivity from 10,000 to 13,000 kg ha⁻¹ (Kim et al., 2007). This technology is used worldwide, especially in Asian countries (Tan, Sun, & Corke, 2002; Kim et al., 2007).

The production of hybrid rice seeds requires a system composed of three lines: male-sterile (line A), male-fertile with the ability to maintain sterility of line A (line B) and another, also male-fertile, line with restored capacity for fertility in line A (line R). The combination of the first two lines (A and B) produces seeds that originate from male-sterile plants (line A seeds). The cross between A and R lines produces hybrid seeds originating from fertile plants (Bragantini, Guimarães, & Cutrim, 2001).

Aiming for success in hybrid seed production, seed A and R lines must be sowed to ensure the synchronization of the flowering of lines, encouraging cross-pollination, which is a crucial factor in the production of hybrid seeds (Mao, Virmani, & Kumar, 1998). High hybrid seed yield from line A is important to reduce seed cost and directly affect the hybrid seed price for the farmers; in Brazil, the average is 500 kg ha⁻¹,

while in China, it is 2,000-2,400 kg ha⁻¹ (Coimbra et al., 2006). According to Peng (2016), worldwide, the hybrid seed yield varies from 1,000 to 3,500 kg ha⁻¹.

Hybrid rice seed production is done in areas with rows or blocks of line R (pollinator) with rows or blocks of line A (receptor) in proportion of 2 (receptor) by 1 (pollinator) or 1 by 1, which results in the high cost of both the hybrid seeds and to commercialize these seeds (Huang, Tang, Ao, & Zou, 2017). According to Virmani and Kumar (2014), the biggest challenges in hybrid rice seed adoption by farmers is connected to the low yield of the hybrid seed fields; furthermore, hybrid seeds can cost 10 times more than traditional rice seeds.

Some alternatives have been used to reduce investment in hybrid rice, such as reducing the amount of hybrid seeds in the sowing operation from 100 kg ha⁻¹ used in conventional fields to 50 kg ha⁻¹ in hybrid fields (Peske & Barros, 2006; Lin, Zhu, Chen, Cheng, & Uphoff, 2009; Badshah, Tu, Zou, Ibrahim, & Wang, 2014; Goulart, Schuch, Tunes, & Vieira, 2015). However, this isolated practice did not provide significant increases in the adoption of hybrid seeds in Brazil (Coimbra et al., 2006; Mondo, Nascente, Neves, Taillebois, & Cardoso Neto, 2016). Other alternatives are being studied, such as the use of lines resistant to herbicides (Zhang, Xu, Wu, & Zhu, 2002) and parental lines with different seed sizes (Zhu, Wang, Ni, & Cheng, 2011) or different seed colors (Maruyama, Kato, & Araki, 1991; Nethra, Rajendra, Vishwanath, & Dhanraj, 2007). Mondo et al. (2016) studied the effects of sowing depth, plant density and fertilization with nitrogen or phosphorus as potential techniques to increase the pollen availability in the field and, consequently, the flowering synchrony between parental lines in the production of hybrid rice seeds. Authors reported that such techniques constitute potential alternatives for use in hybrid rice seed production systems and could be applied in alternated blocks of R lines in the field to obtain longer periods of pollen availability.

Agronomic techniques should be developed in order to improve hybrid seed yield and reduce the cost of production and hybrid seed price for farmers. Our objective was to determine the proportion of seeds of line A (receptor) and line R (pollinator) under mixed sowing that allows optimized rice hybrid seed production and grain quality.

Material and methods

Site description

The experiments were conducted at Lowland Station, Embrapa Temperate Agriculture, located in Capão do Leão county, Rio Grande do Sul State, at 31°48'13" S and 52°24'41" W and 823 m of altitude. The regional climate is Cfa according to the Koppen classification system, humid subtropical with a mean annual temperature of 17.8°C and rainfall of 1,366 mm. The soil was classified as a Typic Albaqualf soil (Soil Survey Staff, 2010).

Experimental design and treatments

Two independent experiments were performed in different areas for two consecutive growing seasons (2015/16 and 2016/17). The cultivar INTA Puita was used as the pollinator and line 464 A as the receptor. The experimental design was a randomized block with four replications. Treatments were six proportion of seeds of INTA Puita (0.75, 1.5, 3.0, 6.0, 12.0 and 24.0%) mixture with seeds of line 464 A (99.25, 98.5, 97.0, 94.0, 88.0, and 76%, respectively). The plots for both trials had a dimension of 5.00 m (25 rice rows) width x 5.40 m length. The usable area of the plots consisted of 20 central rows of rice, disregarding 0.50 m on each side.

Crop management

The experiments were sown on November 26, 2015, and December 3, 2016. The average emergence of rice seedling took place four days after sowing in 2015 and six days after sowing in 2016. The flowering of INTA Puita occurred on February 21, 2016, and January 31, 2017, and the flowering of 464 A occurred on February 20, 2016, and February 2, 2017.

Land was prepared in the conventional way (disking and leveling). The seed density was 50 kg ha⁻¹, the row spacing was 0.20 m, and the seeding rate was 80 viable seeds per meter. The fertilization was carried out based on soil analysis (SOSBAI, 2016). Therefore, at sowing, we applied 90 kg ha⁻¹ of K₂O as potassium chloride, 60 kg ha⁻¹ of P₂O₅ as triple superphosphate and 15 kg ha⁻¹ of N as urea; this was supplemented with an additional 60 kg ha⁻¹ of N as urea at topdressing 20 days after rice emergence plus 30 kg ha⁻¹ of N as urea

at topdressing in the panicle differentiation stage. Cultural practices were performed according to standard recommendations for a rice crop to keep the area free from weeds, diseases and insects. Harvesting was performed manually after physiological maturation separating each material.

Evaluations of traits

Grain yield: The grain yield was determined by weighing the harvested grain of each plot, corrected to 13% of the water content and converted to kg ha^{-1} .

Milling yield: The milling yield was determined by collecting a sample of 100 g of rice grains in each plot, which was processed in a test mill for 1 minute; then, the polished grains were weighed, and the value obtained was regarded as the effective performance, with the results being expressed as a percentage. Subsequently, these polished grains were placed on a grain "Trieur" 2 machine, and the separation of the grains was processed for 30 seconds; the grains remaining in the "Trieur" were weighed to obtain the yield of broken grains, which was also expressed as a percentage.

Cost of production: According to Conab (2018), in the irrigated rice crop system, for a yield of $7,850 \text{ kg ha}^{-1}$, the cost per hectare of conventional seeds was R\$ 6,795.03 in Pelotas (RS) or US\$ 1,727 per ha. The cost of the hybrid seeds was R\$ 7500.03 or US\$ 1,875 per ha (Wander & Padrao, 2017). With this information of costs of conventional and hybrid seeds, we calculated the cost of production for each treatment. With the cost of hybrid production and production of hybrid seeds, we calculated the cost per kg of hybrid seeds for each treatment.

Revenue: The revenue attained from the commercialization of the hybrid + pollinator (INTA Puita CL) was calculated. We considered that we would sell the production as hybrid seeds for the receptor line (line 464 A) and the INTA Puita CL as grain. For this, the price paid for a 50 kg-bag of rice in Pelotas-RS on December 12, 2018, US\$ 10.54 or US\$ 0.21 per kg of rice grain (AGROLINK, 2018) for INTA Puita CL was used. The price used for the hybrid seeds were US \$ 5.00 per kg (Wander & Padrao, 2017).

Statistical analyses

For statistical analysis, the SAS Statistical Software (SAS, 1999) was used. Data were subjected to an analysis of variance, and when the F test proved significant, data were analyzed by regression analysis.

Results

According to our results, increasing the percentage of the pollinator seeds (INTA Puita CL) from 0.75 to 24% in the mixture with the receptor (line 464 A) increased the production of INTA Puita CL grain yield from 7 to 2676 kg ha^{-1} with a linear regression (Figure 1), increased the total production per area (hybrid seed + INTA Puita CL grain) from 286 to 4169 kg ha^{-1} also with a linear regression, and hybrid seed per area from 279 to $1,493 \text{ kg ha}^{-1}$ in a quadratic fashion. The mixture of different proportions of INTA Puita CL (pollinator) with the receptor (line 464 A) did not result in any changes in the grain quality (Figure 2).

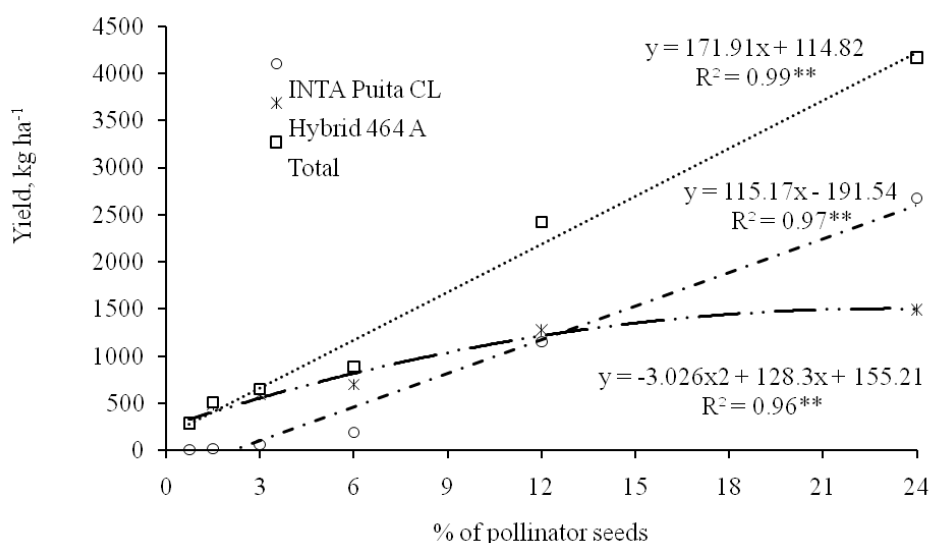


Figure 1. Yield of INTA Puita CL, hybrid 464 A and the total (INTA Puita CL + hybrid 464 A) as a function of the percentage of INTA Puita CL (0.75, 1.5, 3.0, 6.0, 12.0, and 24.0%) mixture with line 464 A (99.25, 98.5, 97.0, 94, 88, and 76%, respectively).

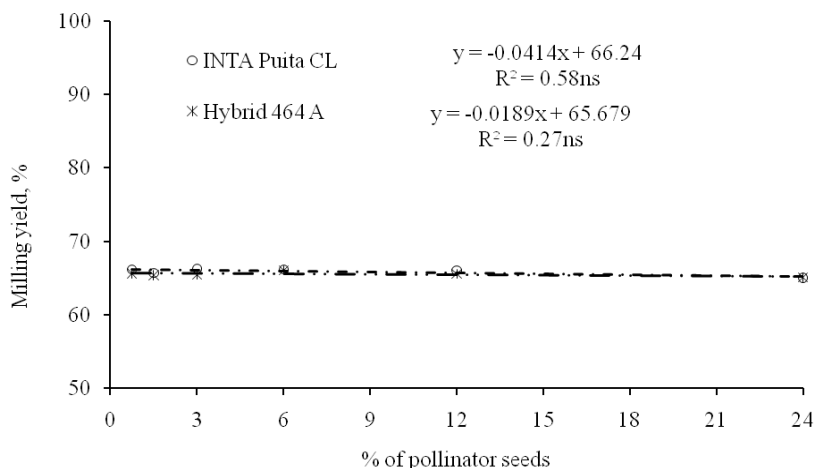


Figure 2. Milling yield of grains of INTA Puita CL and hybrid 464 A as a function of the percentage of INTA Puita CL (0.75, 1.5, 3.0, 6.0, 12.0, and 24.0%) mixture with line 464 A (99.25, 98.5, 97.0, 94, 88, and 76%, respectively).

The analyses of the production cost of a different mixture of INTA Puita CL with line 464 A showed that the price to produce each mixture was very similar to the others (Figure 3). The cost of production per kg of hybrid seeds decreased linearly with increasing percentage of the pollinator (INTA Puita CL), from US\$ 6.71 to US\$ 1.23 (Figure 4).

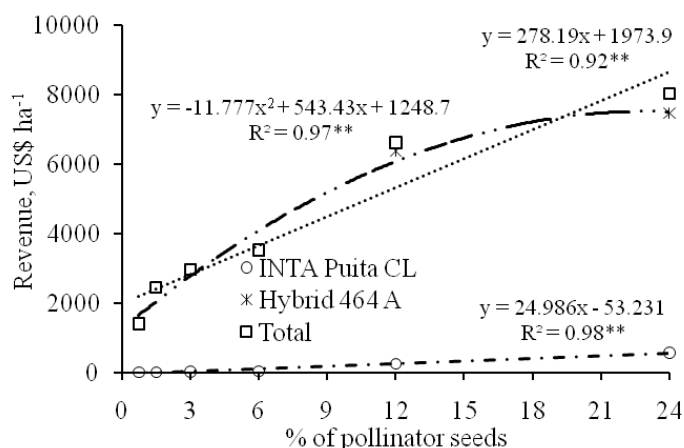


Figure 3. The cost of the production of grains of INTA Puita CL and hybrid 464 A as a function of the percentage of INTA Puita CL (0.75, 1.5, 3.0, 6.0, 12.0, and 24.0%) mixture with line 464 A (99.25, 98.5, 97.0, 94, 88, and 76%, respectively).

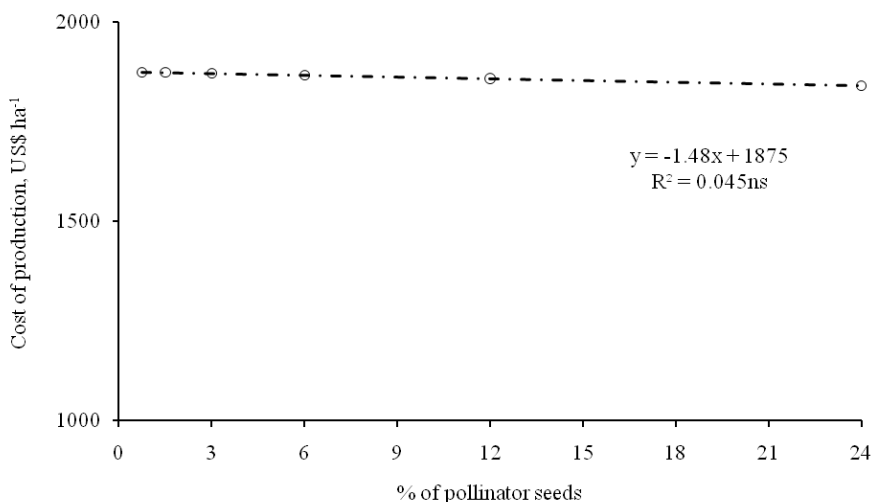


Figure 4. Cost per kg of hybrid seeds as a function of percentage of INTA Puita CL (0.75, 1.5, 3.0, 6.0, 12.0 and 24.0%) mixture with line 464 A (99.25, 98.5, 97.0, 94, 88 and 76%, respectively).

The calculation of the revenue achieved from the commercialization of the grains of INTA Puita CL with hybrid seeds 464 A showed linear increases when increasing the percentage of INTA Puita in the mixture until 24%, with a maximum revenue of US\$ 8650 (Figure 5). In addition, the revenue from the hybrid production increased in a quadratic fashion, with a maximum value of US\$ 7765 at 24% of INTA Puita CL. Revenue from the commercialization of INTA Puita CL grains showed a linear regression with a maximum value of US\$ 562.

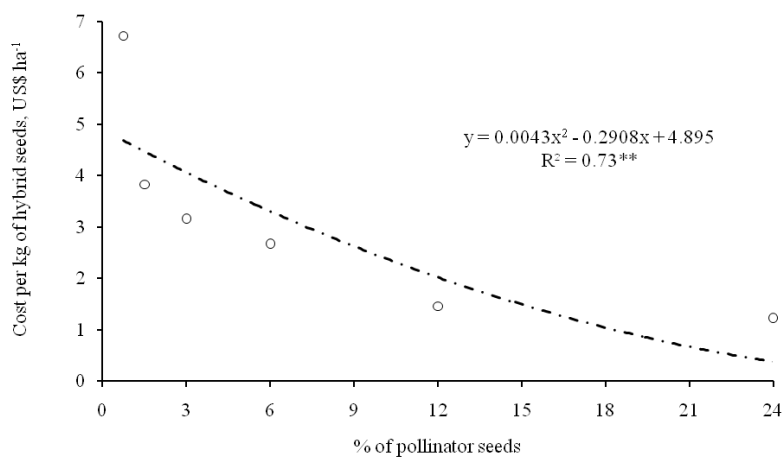


Figure 5. Possible revenue achieved with the commercialization of grains of INTA Puita CL and hybrid 464 A as a function of percentage of INTA Puita CL (0.75, 1.5, 3.0, 6.0, 12.0, and 24.0%) mixture with line 464 A (99.25, 98.5, 97.0, 94, 88, and 76%, respectively).

Discussion

Our results showed that it is possible to improve hybrid production of rice with the technique of increasing the percentage of the pollinator in the field. In the mixture of 24% of the pollinator INTA Puita CL and 76% of the receptor 464 A, we could produce 1,493 kg ha⁻¹ of hybrid seeds (Figure 1). This result is very important and is three times larger than the Brazilian average (Coimbra et al., 2006); worldwide, the hybrid seed yield varies from 1,000 to 3,500 kg ha⁻¹ (Peng, 2016). In addition, this technique allowed the production of 2,676 kg ha⁻¹ of grains from INTA Puita CL that could be sold at a good price, once the mixture of seeds from the two materials did not reduce the grain quality of the rice (Figure 2).

Another important result was that production cost with a different mixture of pollinator and receptor was similar (Figure 3). In this sense, we could infer that the increasing proportion of the pollinator until 24% will not provide increases in the hybrid seed production costs. One of the biggest challenges to improve use of hybrid seed in rice fields by the farmers is related to the cost of these seeds (Virmani & Kumar, 2014).

By increasing the proportion of the pollinator and the yield of hybrid seeds, the cost per kg of hybrid seeds reduced quadratically from US\$ 6.71 to US\$ 1.23 (Figure 4). According to Coimbra et al. (2006), increasing the yield of hybrids directly affects the hybrid seed price for the farmers and can help to increase the adoption of this technology. The cost of conventional rice seeds is around US\$ 1 (CONAB, 2018), whereas that of hybrid seeds is around US\$ 5 (Wander & Padrao, 2017). With the technique used in this research, the cost of producing hybrid rice could reach US\$ 1.23, making it very attractive for hybrid seed producers to produce and sell these seeds and increase the adoption of this technology. According to Virmani and Kumar (2014), hybrid seeds cost much more than traditional rice seeds, which is an important reason for the reduced number of farmers adopting this technology.

With the technique tested in our trial, we see that it is possible to achieve a revenue of US\$ 8,650 (Figure 5) from the commercialization of the grain of INTA Puita CL plus the hybrid seeds of 464 A. Therefore, farmers could improve their profits, and there is a way to reduce the price of the hybrid seeds to make the use of this technology more attractive to farmers.

Reducing the cost of hybrid seeds is one of the main goals of hybrid seeds researchers to help increase the adoption of its use. Many alternatives are being studied worldwide aiming for an increase in the field population of receptor lines that will produce rice hybrid seeds and reduce pollinator lines; this would facilitate the harvesting process and allow only hybrid rice to be harvested or would create mechanisms to

separate hybrid seeds from conventional seeds, such as the use of parental lines that are resistant to herbicide (Zhang et al., 2002), have different seed sizes (Zhu et al., 2011) or colors (Maruyama et al., 1991; Nethra et al., 2007), or have nitrogen or phosphorus-based sowing depth, plant density and fertilization (Mondo et al., 2016). Our results showed a potential technique that could be used to improve hybrid seed production, reduce cost per kg of hybrid seed and improve revenue of producing hybrid seeds. Further research should be done to optimize the harvesting process to only harvest the hybrid seeds, since using our technique does not allow the separate harvesting of the pollinator and hybrid seeds, which could be quite difficult in large areas.

Conclusion

By increasing the percentage of the pollinator seeds (INTA Puita CL) from 0.75 to 24% in the mixture with the receptor (line 464 A), we increased the yield of INTA Puita CL grains and hybrid seeds.

The mixture of different proportions of INTA Puita CL (pollinator) with the receptor (line 464 A) did not yield any changes in the rice grain quality nor the production cost.

The cost of production per kg of hybrid seeds decreased quadratically with the increasing percentage of the pollinator (INTA Puita CL), from US\$ 6.71 to US\$ 1.23.

Revenue achieved from the commercialization of the grains of INTA Puita CL with hybrid seeds 464 A showed quadratic increases when increasing the percentage of INTA Puita in the mixture.

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