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Economic impact of marker-assisted selection and rapid generation advance on breeding programs

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Abstract Plant breeding for the generation of cultivars adapted to local conditions has been an important and strategic concern of developing countries with agriculture-based economies. Considering economic constraints, breeders must improve genetic gain to increase the delivery of better cultivars with lower costs, through the implementation of molecular breeding and rapid generation advance. The aim of this work is to assess the actual economic impact of the implementation of these technologies on genetic gain for yield, rice blast disease resistance, and grain amylose content in a conventional rice breeding program. This analysis is intended as a case study of public breeding programs in developing countries. To accomplish this objective, cost analyses and genetic gain estimations were performed for four rice breeding scenarios: conventional and marker-assisted selection, with and without rapid generation advance. These estimations were then used to develop a cost index reflecting the breeding efficiency. The most efficient method was found to depend on the objective trait considered. For yield, there are small variations in genetic gain, but in terms of costs, the application of technology increases the breeding efficiency. For rice blast resistance, marker-assisted selection is not an efficient option when not using rapid generation advance. Conversely, the efficiency of marker-assisted selection increases when using rapid generation advance. For grain amylose content, the greatest effect on genetic gain is obtained when using marker-assisted selection. Rapid generation advance always increases the breeding efficiency. The use of new technological tools is recommended in terms of the cost–benefit function.

Keywords Breeding efficiency · Cost index · Genetic gain · MAS · RGA

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Introduction

Plant breeding to generate cultivars adapted to local conditions (managements systems, soil types, and climate conditions) has been an important and strategic concern in countries with agriculture-based economies. The importance of the private sector in the seed industry challenges and questions the existence of public breeding programs. A survey of the current development of new cultivars in the public sector in the USA revealed a decline in both investment and breeders (Shelton and Tracy 2017).

Although the prevalence of private companies is indisputable, the genetic products they offer do not necessarily address the needs of small countries with unique conditions in environmental, market, socioeconomic, and institutional terms. In such cases, the only alternative available to farmers may be to sow imported seeds that are not necessarily well adapted to local conditions. This situation challenges politicians and scientists from public institutions, who must support and generate adapted genetics to improve local productivity. To achieve this aim, breeders and biotechnicians must improve genetic gain to increase the delivery of better cultivars with lower costs, considering the economic constraints of developing countries (Cobb et al. 2019).

Uruguay is a small country (176,215 km^2) located in the southeast region of South America. Its economy is mainly based on livestock production and agriculture. Despite bordering Argentina and Brazil, Uruguay's soil, climate conditions, and management systems have conditioned the varieties of different crops used by farmers. Hence, Uruguay is a case study for analyzing the importance of national public breeding programs in developing countries and the need to increase the genetic gain to obtain cultivars adapted to local conditions. In fact, Elliot (2010) emphasized the role of plant breeding in Uruguay's situation, mentioning that "plant breeding, which falls largely in the public sector, is a strategic part of Uruguay's response to changes going on around it."

Uruguay's investment in science and technology, particularly in plant breeding, is marginal when compared with international standards. This low investment has brought into question the actual impact that national breeding programs can achieve. The National Institute of Agricultural Research (INIA) is the main public institution engaged in the development of rice varieties for Uruguayan farmers. INIA's rice breeding program (RBP) is mainly focused on breeding for yield, grain quality, and disease resistance. Despite the low investment in comparison with other developing countries, the local RBP has been very successful, as more than 60% of Uruguayan rice crops are sown with RBP's varieties (MGAP 2018). This success derives from a high efficiency in the use of allocated resources. However, it is necessary to analyze and improve the current breeding pipeline to continuously increase the rate of variety generation.

A plant breeding program is a process which aims to develop new and improved cultivars. To accomplish this aim, breeders must generate genetic variability and perform selection to achieve superior recombination of genetic material (Ceccarelli 2015). Breeding success is mainly determined by the genetic gain, i.e., the selection response by trait represented as the product of the selection differential and the heritability of the trait of interest, obtained via the breeding process. Genetic gain is defined as $\Delta G = i\ h^2\sigma_P$ (the "breeder's equation") and is commonly expressed as a function of time or L ($\Delta G = i\ h^2\sigma_P/L$), where i is the selection intensity, h^2 is the trait heritability, σ_P is the phenotypic variability in the population, expressed as the square root of phenotypic variance (σ_P^2), and L is the breeding cycle measured in years (Xu et al. 2017). To increase ΔG, all variables can be modified, but the selection of the variable and the intensity of the modification depend on the breeding strategy, the cost–benefit relation, and the availability of technology; For instance, to only increase i, the total population size should be increased; to increase h^2, the experimental design and phenotyping methodologies should be optimized, whereas to increase σ_P, genetic diversity should be increased with a concomitant regression in genetic adaptability. On the other hand, decreasing the breeding cycle (L) implies decreasing the time it takes to evaluate, select, and recombine desired breeding material, expressed in years (Brennan et al. 2005, Rutkoski 2019).

In conventional breeding (CB) programs, genetic variability is generated by crossing genotypes with different performance and selection is based on phenotypic information. Breeding programs which integrate molecular tools, in addition to phenotyping, use molecular markers as supplementary genotypic information. INIA's RBP, like most of the world's, is a

CB program based on the pedigree methodology involving single plant selection during segregating generation followed by yield testing (Collard et al. 2017). This program is beginning to use marker-assisted selection (MAS) and rapid generation advance (RGA) to increase selection intensity and reduce the breeding cycle length, respectively. The implementation of both technologies represents, on the one hand, a new challenge in technical terms and, on the other, a great challenge in terms of maintaining the cost efficiency equation, validating future breeding programs in Uruguay.

The analysis presented herein is intended as a case study for public breeding programs to assess the actual economic impact of implementing MAS and RGA in a conventional rice program. To accomplish this objective, cost and ΔG analyses were performed and are discussed in the context of a single average cross. Four breeding scenarios were identified and compared: CB and MAS, with and without RGA (CB + RGA, MAS + RGA). Yield (Y), rice blast disease resistance (RBDR), and grain amylose content (AC) were the target traits selected in all scenarios. MAS was only considered for RBDR and AC.

Materials and methods

Breeding scenarios analyzed in this study

Four breeding scenarios were compared in terms of ΔG and cost. Figure 1 describes the CB scenario, showing the breeding location (field or greenhouse), generation (F1–F11), selection unit (plant, family, line), number of selection units, experimental unit (crossing pod, breeding row, phytopathology row, laboratory analysis), and selection criteria. All other scenarios are described in ESM_1, ESM2, and ESM_3. Although the main breeding objective is yield increase (yield based selection, YBS), RBDR and high AC are also important selection objectives. RBDR was evaluated in an infection chamber in a greenhouse during the F3 generation, and in an infection nursery in the field in F9 and F10. RBDR was evaluated using a pathogenicity rating scale developed by IRRI (1996). AC percentage in F9 and F10 was determined by laboratory analysis. All this information was obtained from actual breeding procedures carried out as part of INIA's RBP. All other scenarios were simulated based on real CB data and INIA's current capabilities, specifically genotyping for MAS and greenhouse infrastructure for RGA.

The simulation was based on the following considerations: in MAS scenarios, molecular marker information is used for selecting F2 offspring with favorable alleles of one gene for RBDR and another for AC. RBDR and AC are two required traits for a breeder to meet market demand (Cobb et al. 2019). Five thousand F2 seeds were sown in seedling trays (100 plants/tray), genotyped during early vegetative stages, and transplanted to the field after selection by genotyping. Considering two traits and mendelian segregation, 1/16 of the F2 population was selected (312 F2 plants). Afterwards, the breeding process was the same as described for CB (Fig. 1). In fact, the same amount of plants was maintained in the following generations.

Genotyping included leaf sampling, DNA extraction, DNA amplification, marker detection, and reporting of results. One SNP per trait was used. SNPs were amplified and detected using KASP technology (Semagn et al. 2013).

Currently, INIA's RBP performs only one generation per year. In this study, RGA alternatives with two generations per year were included as alternative scenarios (CB + RGA, MAS + RGA). In RGA breeding, the single seed descent (SSD) instead of classical pedigree method is used (Collard et al. 2017). When SSD is used, lines are fixed (making them homozygous) during early generations (F3 to F6) without single plant selection based on breeder's eye criteria. However, RBDR selection could be done by challenging the plants in infection chambers. The only difference between these two RGA scenarios is that, in the MAS + RGA scenario, plants are selected by genotyping and only six plants are maintained in seedling trays.

The total amount of selection units (SU) and experimental units (EU) are described in ESM_4.

In INIA's RBP organization, all CB activities, RBDR phenotyping (infection chamber and infection nursery), and AC laboratory analysis are carried out at the Treinta y Tres Experimental Station. On the other hand, RGA and genotyping activities are done in greenhouses and laboratories located at Wilson Ferreira Aldunate Experimental Station. This clarification is necessary when analyzing costs.

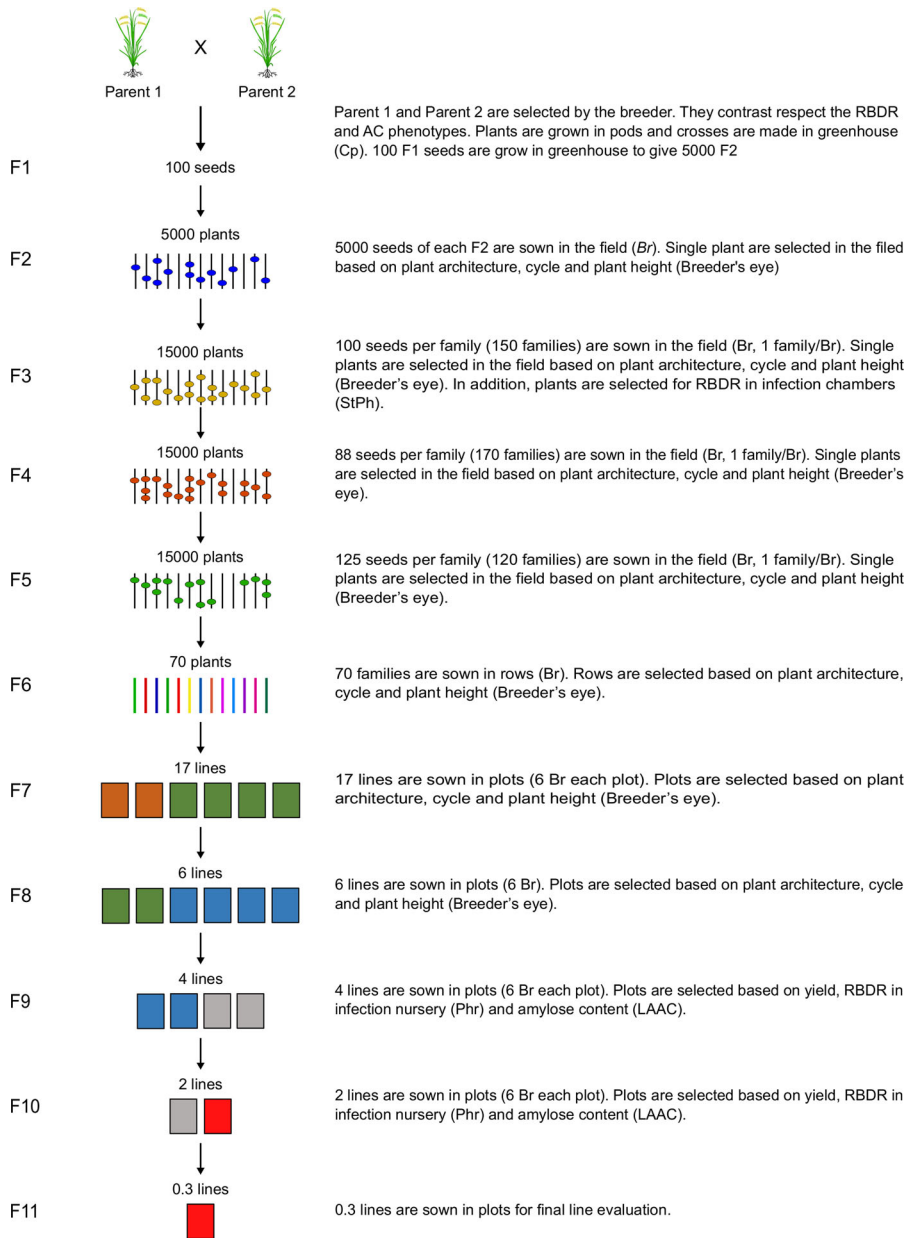

Fig. 1 Description of INIA's current conventional rice breeding program. Breeding location (field or greenhouse), generation, selection unit (plant, family, line), number of selection units, experimental unit (crossing pod, breeding row, phytopathology row, laboratory analysis), and selection criteria for each breeding generation. AC, amylose content; Br, breeding row; Cp, crossing pod; LAAC, laboratory analysis amylose content; Phr, phytopathology row; RBDR, rice blast disease resistance; SNP, single-nucleotide polymorphism; StPh, seedling tray phytopathology

Estimation of ΔG

For each trait, ΔG was estimated each year as $\Delta G = ih^2\ \sigma_P$, where i is the selection intensity, h^2 is the trait heritability defined as an additive variance (σ_A^2) over σ_P^2, where σ_P is the phenotypic variability in the population expressed as the square root of σ_P^2.

Selection intensity was calculated using the real selection proportion used in the current CB scenario (Acquaah 2007). A selection intensity calculator table was used (https://jvanderw.une.edu.au/) (Oldenbroek and van der Waaij 2015). For the σ_P^2 estimation, real phenotypic information from the F6 generation was obtained from *tropical japonica* germplasm, during 2003, 2004, 2006, 2013, and 2017. This σ_P^2 was calculated as the average of σ_P^2 for lines derived from one cross, considering families with 20 or more lines. σ_P^2 for F2 to F10 was calculated by applying the change in σ_P^2 obtained from the selection

intensity calculator table (https://jvanderw.une.edu.au/).

For yield, the same set of families were used to calculate h^2. From F2 to F5, an h^2 of 0 was assumed for breeder's eye. This assumption was taken, considering that the h^2 of the indirect trait (yield) is equal to the h^2 of the direct trait (plant height and plant cycle) multiplied by the correlation between them. Although in more diverse germplasms plant cycle and architecture can be highly correlated with yield, when diversity is narrowed by the process of adaptation and prebreeding, as is the case, the correlation is very low (near zero). For RBDR and AC, h^2 was calculated as the average of h^2 for each family, each estimated in a joint model with all the years and trials available for that family.

To calculate the ΔG for the complete breeding cycle, the annual ΔG was added and divided by the length of the cycle interval (L), expressed in years.

For the MAS scenario, the following assumptions and calculations were made: (1) the trait h^2 is equal to the marker h^2, and equal to 1. (2) For RBDR, σ^2_P can be estimated by assuming a resistant parent phenotypic value = 0 and a susceptible parent phenotypic value = 4. (3) For AC, σ^2_P can be estimated as the average of the real lowest (15.17%) and highest (21.42%) phenotypic average in CB obtained from families evaluated in yield trials (YT).

Cost analysis used in this study

To facilitate correct understanding of this study, classical cost parameters are defined in ESM_5.

Genetic gain was selected as a target cost, as it allows comparison of breeding scenarios in terms of breeding process quality and quantity, providing a measure of the progress generated. In this work, a complete costing model was used to compare all scenarios, since they involve different productive factors, in relation to the selected target cost.

The intermediate product in the cost analysis was the experimental unit (EU). Considering INIA's RBP organization, the EU cost depends on the breeding strategy, stage, selection target trait, and breeding location. According to INIA's RBP organization, the activities are organized as follows: (1) breeding per se, which involves crosses in special greenhouses and conventional field activities (multiplication and evaluation); (2) selection for AC, involving only AC laboratory activities; (3) RBDR breeding, which involves evaluation in an infection chamber in a greenhouse and in an infection nursery in the field; (4) molecular markers including genotyping activities in a biotechnology laboratory; (5) RGA performed in a biotechnology greenhouse. As mentioned above, all rice breeding, rice quality, and phytopathology activities are carried out at Treinta y Tres Experimental Station, while biotechnology activities are carried out at Wilson Ferreira Aldunate Experimental Station. Depending on this criterion, the following EU were defined: breeding rows (Br) (one field plot being formed by six breeding rows), crossing pod (Cp), AC laboratory analysis (LAAC), phytopathology seedling tray (StPh) for RBDR infection chamber tests, phytopathology rows (Phr) for RBDR infection nursery in the field, laboratory analysis biotechnology for genotyping (LABio), and seedling tray biotechnology (StBio) for RGA.

The number of total EU (NTEU) depends on the amount of selection units (SU), which are single plants (Sp) or fixed lines (FL), depending on the breeding stage. For instance, for the RGA scenario in F2, there are 100 single plants (SU) arranged in two seedling trays (EU) of 50 plants each. Since there is only one repetition (R) and environment (E), the NTEU is 2 (100/50). The same line of reasoning is applied throughout the analysis for all generations (from F1 to F10) and in all scenarios (ESM_4).

For MAS scenarios, to facilitate the cost analysis, the EU were separated as MAS per se (involving breeding seedling trays and genotyping) and yield base selection (involving the subsequent CB-like activities). This separation was useful for cost estimation, since both procedures are separated in the field and the laboratory. In the case of MAS + RGA, there is only one type of EU, since plants are maintained in seedling trays. In MAS scenarios, RBDR and AC costs are assigned to MAS cost, since it is the only selection criterion for these traits.

Considering each breeding scenario as a standard process, a process costing system was chosen. However, for specific laboratory activities (RBDR infection chamber and AC determination) an activity-based cost was used (Kaplan and Cooper 2003). This costing system for laboratory activities was chosen because this laboratory currently performs multiple analyses and costs are not recorded separately.

Depending on the breeding scenario, a resultant or standard costing model was used (ESM_5). For the CB scenario (INIA's current rice breeding method), the resultant costing model was used. For all other scenarios, which are simulated, a standard costing model was used. The two models are comparable since the standard costing was developed using actual INIA efficiency parameters.

For the cost analysis, INIA's 2017 accounting databases in dollars (US$) were used. These databases are classified, according to their nature and economic participation in the value-generating processes, into: (1) consumable materials in the first use, (2) workforce, (3) intermediate services, (4) financial resources, (5) material consumer goods, and (6) natural resources. Once the costs databases for the rice CB process were obtained, they were classified using the variability and traceability methods for correct cost allocation to the target costs. The indirect cost distribution bases were selected based on the nature of the cost factors to be traced (ESM_6).

RGA allows shortening of the breeding cycle. Hence, RGA generates an opportunity cost by getting a new rice variety to market earlier. For this study, the opportunity cost was calculated as the cost of investing funds in carrying out or increasing breeding processes. For the comparison of scenarios at each cost, this estimation simplifies the equation, since it is multiplied by the time (L) that it demands.

For a comparative cost measurement, the opportunity cost of the time invested in each scenario should be considered. As an alternative, for the objective of equalizing the unit of measurement to compare the different scenarios, a cost index was created as the quotient between the total cost of each scenario and the average annual ΔG. Therefore, this index is a comparative measure of the effort required to generate a unit of ΔG for each scenario. Thus, the ΔG of equal periods is measured in equivalent terms, penalizing those derived from scenarios of major selection cycles.

Results

Cost analysis for four breeding scenarios

Considering the organization of the work in INIA's RBP, the EU depends on the breeding activity but also on the breeding location. Table 1 presents the variable and fixed costs for each EU calculated in this study (breeding rows, crossing pod, laboratory analysis for AC, seedling trays for phytopathology, and biotechnology laboratory analysis), which are necessary for calculating the total cost. The structure of the table shows that the EU depends on the activity. In turn, the activity is related to the physical structure where it takes place (field, laboratory, or hothouse). The scenarios are then structured according to the number of experimental units they consume from each activity.

For cost analysis, it was necessary to determine the NTEU for each breeding generation (F1 to F10) and trait in all scenarios, based on current real data from the CB scenario (Tables 2 and 3). As described in "Materials and methods" section, since the number of selection units in CB and MAS was maintained, the NTEU (YBS) in both scenarios is the same (Table 2). The difference is found for the RBDR and AC traits. In the CB scenario, the phenotypic selection for these variables is carried out in F2, F9, and F10 for RBDR, and in F9 and F10 for AC. In the case of MAS, the selection is made only in F2 using molecular markers. The 50 NTEU (RBDR and AC) of the MAS scenario refers only to the StBio. The number of genotyped samples in MAS scenarios (5000 samples) is not shown in this table. In the case of the RGA scenarios (Table 3), since the plants are in StBio, no additional cost is required in the MAS scenario beyond the genotyping costs, which are not shown in Table 1. In the MAS + RGA scenario, the number of genotyped plants is 100, which is significantly lower than the 5000 in the MAS scenario.

The biggest differences occur when comparing the RGA scenarios with those that do not implement RGA. In fact, the fundamental difference is given by the decrease in the number of selection units when using RGA due to the use of SSD. In the case of MAS + RGA, contrary to MAS, there is no difference between the NTEU for YBS and the NTEU for RBDR and AC. This occurs since, when the plants are kept in seedling trays after being selected by markers, costs associated with them are not generated, beyond the genotyping costs that are not shown in Table 1.

ΔG in each breeding scenario and relation to cost

ΔG was calculated for each generation in all breeding scenarios (Table 4, ESM_4). The ΔG of the total

Table 1 Cost analysis for experimental unit in relation to breeding organization (location and strategy)

	YBS		AC	RBDR		MM	RGA
EU	Br	Cp	LAAC	StPh	Phr	LABio	StBio
Variable costs							
General expenses	27,352	456	7978	365	1459	2500	975
Experimental field	113,223						
Laboratory			3304	1666	6665	37,901	
Greenhouse		328		262	1050		5494
Workforce direct cost	105,699	22	25,362	13,693	9129	28,905	10,007
Workforce indirect cost	218,625	18,219	63,765				
Fixed costs							
Laboratory			15,565	67	2798	12,889	65
Greenhouse		1483		3503			
Experimental fields	2470						
Other indirect costs							
Experimental fields	31,528						
Machinery services	53,883						
General cost (EE)	166,542	2776	48,575	2221	8882	17,073	3456
Total cost	719,322	23,283	164,548	21,777	29,983	99,269	19,997
Total of current EU	123,810	50	2300	264	4000	80,640	429
Ct/EU	5.81	465.66	71.54	82.49	7.50	1.23	46.66

AC, amylose content; Br, breeding row; Cp, crossing pod; Ct, costs; EU, experimental unit; LAAC, laboratory analysis amylose content; LABio, laboratory analysis biotechnology; MM, molecular markers; Phr, phytopathology row; RBDR, rice blast disease resistance; StBio, seedling tray biotechnology; StPh, seedling tray phytopathology; YBS, yield-based selection

Table 2 Number of total experimental units for scenarios without RGA

Gen.	Year	CB				MAS		
		NTEU (YBS)	NTEU (RBDR)	NTEU (AC)	Costs	NTEU (YBS)	NTEU (RBDR, AC)	Costs
Parentals	0	1	–	–	465.7	1	0	465.7
F1	1	2	0	0	14.3	2	0	14.3
F2	2	75	0	0	535.5	75	50	16,457.8
F3	3	150	13	0	2022.8	150	0	1071
F4	4	170	0	0	1213.8	170	0	1213.8
F5	5	120	0	0	856.8	120	0	856.8
F6	6	70	0	0	499.8	70	0	499.8
F7	7	204	0	0	1456.6	204	0	1456.6
F8	8	72	0	0	514.1	72	0	514.1
F9	9	72	1	8	1116.4	72	0	514.1
F10	10	36	1	4	558.2	36	0	257

AC, amylose content; CB, conventional breeding; MAS, marker-assisted selection; NTEU, number of total experimental units; RBDR, rice blast disease resistance; YBS, yield-based selection

breeding process was calculated as the sum of the ΔG obtained each year, expressed as a function of time (L) due to the importance of speeding up variety delivery.

For RBDR, the use of molecular markers for selection always increases the ΔG, since it is a qualitative trait (decreasing -5.6 leads to complete resistance). However, the use of MAS always increases the cost per line. The major reduction is

Table 3 Number of total experimental units for scenarios with RGA

Gen.	Year	CB + RGA				MAS + RGA	
		NTEU (YBS)	NTEU (RBDR)	NTEU (AC)	Costs	NTEU (YBS, RBDR, AC)	Costs
Parents	1	1	0	0	465.7	1	465.7
F1	1	1	0	0	46.7	1	46.7
F2	1	1	0	0	46.7	0.06	248.8
F3	2	1	0	0	46.7	0.06	2.8
F4	2	1	0	0	46.7	0.06	2.8
F5	2	1	0	0	46.7	0.06	2.8
F6	3	2	1	0	14.3	0.12	0.9
F7	4	204	0	0	1456.6	25.41	181.4
F8	5	72	0	0	514.1	16.94	121.0
F9	6	72	12	4	890.2	12.71	90.7
F10	7	36	6	2	445.1	1.91	13.6

AC, amylose content; CB, conventional breeding; MAS, marker-assisted selection; NTEU, number of total experimental units; RBDR, rice blast disease resistance; RGA, rapid generation advance; YBS, yield-based selection

Table 4 Comparison of ΔG and cost index among four breeding ΔG scenarios

	YBS (kg/ha)	RBDR (IRRI scale)	AC (%)
CB			
ΔG	1401.2	−2.3	0.4
$\Delta G/L$	127.4	−0.2	0.03
Cost.$L/\Delta G$	47.9	4743.1	21,801.3
Cost.$L/\Delta G$/Line	159.8	15,810.2	72,670.9
MAS			
ΔG	1401.2	−4.7	8.8
$\Delta G/L$	127.4	−0.4	0.8
Cost.$L/\Delta G$	44.7	17,107.5	9193.8
Cost.$L/\Delta G$/Line	149.1	57,025.1	30,646.1
CB + RGA			
ΔG	1694.4	−1.7	0.4
$\Delta G/L$	242.1	−0.2	0.1
Ct$L/\Delta G$	12.2	558.8	6735.8
Cost.$L/\Delta G$/Line	40.5	1862.7	22,452.5
MAS + RGA			
ΔG	1159.4	−4.7	8.8
$\Delta G/L$	165.6	−0.2	1.3
Cost.$L/\Delta G$	5.2	542.1	98.3
Cost.$L/\Delta G$/Line	48.8	5119.5	928.7

ΔG, genetic gain; AC, amylose content; CB, conventional breeding; L, time expressed in years; MAS, marker-assisted selection; RGA, rapid generation advance; RBDR, rice blast disease resistance; YBS, yield-based selection

achieved when RGA is used in the breeding process. Hence, the most advantageous scenario is CB + RGA.

These results contrast with those regarding AC trait selection; AC marker selection reduces the cost index more than a half, and almost 200 times when combined with RGA marker selection.

When comparing the cost index developed in this study, Ct$L/\Delta G$, for YBS, application of RGA alone reduces by almost 4 times the cost required to increase ΔG by a given amount. The reduction in this index is related to a decrease in L. The use of MAS, without RGA, does not affect this index. In fact, since MAS is not intended to increase yield, and the population size is maintained, there is no difference in ΔG for YBS trait between MAS and CB. When MAS is combined with RGA, the reduction in the total amount of EU due to marker selection more than compensates the lower ΔG, therefore the cost index is the lowest. The reduction in the population size can be visualized by comparing the parameter *CtL/ΔG/Line*, which captures the population size required for a generation of one final fixed line. When comparing this value, the CB + RGA scenario showed the most positive results, even compared with MAS + RGA.

Considering a yield mean value for INIA's experimental results of 8090 kg/ha, the % ΔG in the total CB and MAS cycle was 17.32% and the % ΔG/mean/year was 1.57. In the case of RBDR and AC, these values were significantly higher for MAS compared with CB. The mean value was 1.48% for RBDR and 18.58% for AC. In the case of CB, the % ΔG/mean/year for RBDR was 14.21, while for MAS it was 28.91. In the case of AC, CB % ΔG/mean/year was very low, only 0.21%, and 4.28% for MAS. The comparison of each scenario with mean values obtained at INIA's experimental field is presented in Table 5.

Table 5 Genetic gain expressed as a proportion of phenotypic mean

Trait	Mean	% ΔG/mean	% ΔG/mean/L
CB			
Yield (kg/ha)	8090	17.32	1.57
RBDR (IRRI scale)	1.4793	156.27	14.21
AC (%)	18.58	2.33	0.21
MAS			
Yield (kg/ha)	8090	17.32	1.57
RBDR (IRRI scale)	1.4793	378.56	34.41
AC (%)	18.58	47.11	4.28
CB + RGA			
Yield (kg/ha)	8090	20.94	2.62
RBDR (IRRI scale)	1.4793	114.32	14.36
AC (%)	18.58	2.40	0.30
MAS + RGA			
Yield (kg/ha)	8090	14.33	1.79
RBDR (IRRI scale)	1.4793	378.56	47.32
AC (%)	18.58	47.12	5.89

ΔG, genetic gain; AC, amylose content; CB, conventional breeding; L, time expressed in years; MAS, marker-assisted selection; RGA, rapid generation advance; RBDR, rice blast disease resistance; YBS, yield-based selection

Discussion

The application of any new technology in a plant breeding program requires a cost analysis justifying its incorporation. Despite the importance of this type of analysis, there are few studies that report cost analyses for the implementation of MAS or RGA and evaluate the application of both technologies (Collard et al. 2017; Slater et al. 2013). Recently, a cost analysis between conventional MAS and a modified-MAS strategies was reported by Arbelaez et al. (2019).

To accomplish a cost analysis, the most important factor is the selection of the suitable target cost to achieve correct decisions regarding the case under study. In this work, the total ΔG per trait was chosen as the target cost since it is a homogeneous variable enabling the comparison of all breeding scenarios. In addition, using ΔG makes it possible to perform the comparison every year during the complete breeding cycle. It was not the aim of this study to quantify the costs to increase RBDR, yield, or AC traits. Rather, the aim is to compare the cost efficiency associated with different breeding scenarios. Hence, the determination of the cost index, which is the quotient between the total cost and ΔG, was one of the main results of this study. This index is a homogeneous tool, useful to compare different breeding technologies. On the other hand, minimizing the cost index per rice line generates a parameter which contemplates the three fundamentals aspects of variety generation, namely generating more lines with more ΔG (better lines) and lowest cost. The scenario that presents the lowest cost index per line will be the one that best meets these three requirements.

In relation to cost determination, the full cost model is essential since it includes fixed costs that are an important differential aspect of each scenario and, therefore, essential to reach a correct valuation and

comparison of them. According to our databases, a great proportion of costs did not have a direct relationship with the target cost considering the established breeding scenarios. The establishment of multiple allocation bases for indirect costs was required, which implied a complex process and a subjective component in the selection. The bases of distribution of indirect costs were selected with criteria of reasonableness to allow an adequate linkage and a correct valuation of the factor in relation to the target cost, with the assumptions of subjectivity underlying this methodology.

Another element to consider when classifying costs, given the nature of the study, refers to the implicit costs, those hidden costs that occur due to the nature of production, such as the impact of the type of production on the environment. The variable time for a new variety to reach the market may entail an implicit opportunity cost, which was not considered for this study. If this cost had been considered, surely the scenarios that reduce the breeding cycles (RGA) and ensure a specific result sought (MAS) would have benefited greatly.

The ΔG values for yield showed that there is small variation among scenarios. It should be mentioned that the yield's ΔG in the MAS scenario, with respect to CB, is overestimated, since the same number of individuals was maintained, but from fewer families. Therefore, σ_P^2 was reduced. It was not possible to address this limitation due to the inability to estimate the phenotypic reduction associated with the reduction of the number of families evaluated in MAS. It is noteworthy that, under the assumptions applied in this study, using MAS for RBDR and AC selection does not affect the yield's ΔG. On the other hand, the yield's ΔG in CB + RGA was higher than in CB. One possible explanation is that, by using SSD in RGA, the genetic variance of F2, which is the maximum, is maintained until the moment when selection can be made with heritability greater than zero. Selecting in the CB scenario with heritability close to zero in early generations erodes σ_P^2 and reduces ΔG.

Differences in ΔG for RBDR between scenarios with and without MAS are due to a higher σ_P^2 in the former. For MAS scenarios, σ_P^2 was estimated from two parents with contrasting phenotype and polymorphism in one major effect resistance gene (sine qua non conditions for successful application of MAS). On the other hand, the σ_P^2 for the CB scenarios was obtained from data observed in the actual breeding populations. The same happens for the AC trait, where the low ΔG in the scenarios without MAS is mainly due to the low σ_P^2 (low variability) for this trait in the local germplasm.

Considering the cost index and the cost index corrected by the number of lines in all cases, except for RBDR, the application of new technologies increases the breeding efficiency. In the case of RBDR, MAS is not an efficient option when not using RGA, but it is cheaper to select for RBDR under field and greenhouse conditions. Conversely, when using RGA, ΔG increases by one order of magnitude. For AC, considering the high cost of AC laboratory analysis, the greatest effect on ΔG is obtained when MAS is used, regardless of the application of RGA. However, RGA further increases the cost-efficiency equation. For yield, CB + RGA is the best scenario in terms of cost and ΔG efficiency.

Our work shows that the cost-efficiency of the application of MAS is dependent on the trait to be improved. On the other hand, the application of RGA is always cost-effective, with significant differences in the cost index in relation to CB. These results agree with those reported by Cobb et al. (2019), who highlighted that cycle time is the parameter that most affects ΔG. Our study shows, in a real situation, that cost-efficiency in terms of ΔG, between scenarios with and without RGA, justifies the investment in facilities (greenhouses) when there are no installed capacities.

In conclusion, although the values depend on the trait to be selected, in all cases, the cost-efficiency increases with the joint application of new tools (RGA and MAS). RGA always increases the efficiency improvement.

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