

Selection of experimental strawberry (*Fragaria x ananassa*) hybrids based on selection indices

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Genet. Mol. Res. 16 (1): gmr16019052

Received August 2, 2016

Accepted December 12, 2016

Published March 8, 2017

DOI <http://dx.doi.org/10.4238/gmr16019052>

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ABSTRACT. The strawberry (*Fragaria x ananassa* Dutch.), is the only vegetable belonging to the rosacea family. All strawberry species have now emerged from wild species and belong to the genus *Fragaria*, being that this genus presents more than 45 described species, and only 11 are considered natural species. Due to the octoploid nature of strawberry and its variability after hybridization, selecting one or more characters may result in unfavorable genotypes and even the exclusion of promising ones, because negative genetic correlations have been observed among them that cause inefficient selection. Therefore, the

objective of this study was to verify the efficiency of selection indices in selecting experimental strawberry hybrids for *in natura* consumption and processing. Seven commercial cultivars and 103 hybrids were used, which were obtained from populations derived from their crossings. The experiment was conducted in augmented blocks, in which four agronomical traits (total mass, amount of commercial fruit, amount of noncommercial fruit, and average fruit mass) and seven physical-chemical traits (soluble solids, soluble solids:titratable acidity ratio, total sugars, total pectin, vigor, and internal and external coloration) were evaluated. For hybrid selection, the following indices were used: Mulamba and Mock (1978), Smith (1936), Hazel (1943), and genotype-ideotype, which selected 20% of the genotypes evaluated. The three indices selected about 9% of the hybrids. The selection of two experimental hybrids (89 and 495) and the use of selection indices resulted in larger estimates of selection gains. The Mulamba and Mock (1978), Smith (1936), and Hazel (1943) indices had the highest percentage of gains on selection, and are therefore recommended for the selection of strawberry clones.

Key words: Strawberry; Selection index; Agronomical trait; Experimental hybrid; Plant breeding

INTRODUCTION

Currently, the main strawberry (*Fragaria x ananassa*) cultivar alternatives that are grown in Brazil are imported, making strawberry farmers dependent on genetic material from other countries (Barneche and Bonow, 2012). This factor represents more than 40% of the production costs, considering the high prices paid for them (about US\$20,000 per thousand). The small number of adapted cultivars that is available to Brazilian farmers has been one of the main obstacles to the development of the strawberry crop, particularly due to the lack of productive cultivars that are resistant to disease and adapted to the climatic conditions of the farming regions (Barneche and Bonow, 2012).

Therefore, the need for the establishment of national programs aimed at the improvement of strawberry is self-evident. Cultivars that are adapted to Brazilian growing conditions need to be identified, in order to improve a series of market traits such as appearance, taste, texture, nutritional value, and high-production potential, as well as resistance to disease. The quality attributes of strawberries destined for *in natura* consumption are taste, appearance, nutritional value, firmness, and total and commercial production. Taste is one of the most important aspects of quality, and is a balance between acidity and sugar (Reis et al., 2013). In cultivars that are destined for processing, the main traits evaluated are °Brix, acidity, productivity, and the anthocyanin concentration.

For the genetic improvement of plants, the evaluation of production is essential, and, for fruit and vegetables, quality is fundamental for the acceptance of the product by the consumer. The use of selection indices in strawberry improvement programs is vital for the simultaneous selection of fruit quality and production attributes (Vilarinho et al., 2003). Fruit quality may comprise size/format, taste, flavor, and coloration, among other traits.

Because of strawberry's octoploid nature and the variability observed in strawberry genetic improvement programs, selection based on one or a few traits may result in unfavorable changes in others, due to negative genetic correlations between them (Hancock et al., 2008). Therefore, it is necessary to employ methodologies that allow selection based on a set of variables that include various traits of economic interest (Cruz et al., 2014). Selection indices, which were initially proposed by Smith (1936) and Hazel (1943), select for a variety of traits simultaneously, which increases the efficiency of selecting promising genotypes.

Many selection indices are used in the improvement of plants (Cruz et al., 2014), and are based on linear combinations of the measurements of several traits. They facilitate the evaluation of all of the information available, and assign different weights to each trait studied and evaluate important quality and production attributes (Falconer and Mackay, 1997).

The selection indices proposed by Smith (1936), Hazel (1943), Williams (1962), and Pesek and Baker (1969) are all parametric, because they require population parameter estimates and consequently are used when genotypes are randomly sampled. The indices of Elston (1963) and Mulamba and Mock (1978) and the genotype/ideotype index are nonparametric, and do not require parameter estimates.

Although they have been used for many crops (Silva et al., 2009; Vasconcelos et al., 2010; Neves et al., 2011), selection indices have not been applied for strawberries, and selection based on independent elimination levels has been conducted.

In some species with vegetative propagation, such as the potato (*Solanum tuberosum* L.) and eucalyptus (*Eucalyptus grandis*), parametric (Smith, 1936; Hazel, 1943) and nonparametric (Mulamba and Mock, 1978) indices have been used for the selection of superior genotypes. Mulamba and Mock's (1978) index has been used for eucalyptus (Reis et al., 2015), soybean (*Glycine max*) (Costa et al., 2004), cowpea (*Vigna unguiculata*) (Santos and Araújo, 2001), and potato (*S. tuberosum*) (Barbosa and Pinto, 1998). The objective of this study was to compare the efficiencies of different selection indices in selecting experimental strawberry hybrids for *in natura* consumption and processing.

MATERIAL AND METHODS

Hybrid development

Genitors were selected that had been cultivated in Brazil and were based on favorable phenotypes in terms of traits of economic interest, and were the 'Aromas', 'Camarosa', 'Dover', 'Florida Festival', 'Oso Grande', 'Sweet Charlie', and 'Milsei Tudla' cultivars. Only 'Aromas' is classified as a day-neutral cultivar, with the others being short-day cultivars.

Hybridization was conducted according to the Campinas Agronomic Institute's (Camargo and Passos, 1993) and the University of Florida's (Chandler et al., 2012) recommended procedures. Twelve hybrid populations were obtained, which combined seven commercial cultivars (Table 1). After harvesting the pseudofruits, the achenes were removed with a blender and the seeds were dried at temperature about 19°-23°C.

The method described by Galvão et al. (2014) was used to overcome tegument dormancy by acid scarification, with immersion in H₂SO₄ (98%) for 40 min and sanitization in NaOH (2%) for 10 min (Galvão et al., 2014). The achenes were then *in vitro*-cultivated in Murashige and Skoog culture (Murashige and Skoog, 1962) that was solidified with agar (0.6%) and supplemented with sucrose (3%).

Table 1. Description of 12 hybrid populations generated from seven strawberry cultivars.

Population	Parents		Population	Parents	
	♀	♂		♀	♂
1	Dover	Aromas	7	Sweet Ch.	Aromas
2	Oso Gr.	Aromas	8	Tudla	Aromas
3	Camarosa	Aromas	9	Tudla	Sweet Ch.
4	Dover	Sweet Ch.	10	Camarosa	Sweet Ch.
5	Oso Gr.	Tudla	11	Festival	Aromas
6	Festival	Sweet Ch.	12	Oso Grande	Sweet Ch.

After 60 days of *in vitro* cultivation, the seedlings were acclimatized in a greenhouse on 72-cell trays containing commercial substrate. After 60 days, the seedlings were transplanted to the greenhouses.

Experimental area

The experiment was conducted in greenhouses, in the experimental area of the Olericulture sector of the Federal University of Lavras, Lavras, MG, Brazil (21°14'S, 40°17'W, 918.80 m above mean sea level). The soil in the experimental area was a typical dystrophic red latosol with a clay texture, and comprised 33% sand, 18% silt, and 49% clay. The soil was prepared in the greenhouses a month before transplantation of the seedlings into beds by plowing, followed by liming and harrowing.

The beds were raised 0.20 m high and 1.20 m wide using a bed tiller. We used 2.50 t·ha⁻¹ lime (Relative Total Neutralization Power 92%), which was based on a chemical analysis of the soil. The seedlings' dry leaves were removed, and they were then transplanted into beds in an area of 0.30 m x 0.40 m in two lines. The irrigation system consisted of drip lines, with 0.30 m between drippers, two lines of drip per bed, separated by 0.50 m. The beds were covered with 30- μ m-thick black polyethylene films (mulching). Fertilization and cultivation followed the recommendations made by Dias et al. (2007) for strawberry crops. Weed control was conducted manually every 15 days, or as needed. Phytopathogen control was performed every two weeks or as necessary, using insecticides and fungicides that were provided by Universidade Federal de Lavras.

The experimental design was an augmented block (Federer, 1956), which was chosen due to a lack of genotype replications, because the object under study was the F1 generation that only had one plant per treatment. Therefore, the common treatments were the parents (controls, 'Aromas', 'Camarosa', 'Dover', 'Florida Festival', 'Oso Grande', 'Sweet Charlie', and 'Milsei Tudla') and nine F1 experimental hybrids from seven populations (9 hybrids x 7 populations = 63 hybrid/plant), plus 10 F1 experimental hybrids from five populations (10 hybrids x 5 populations = 50 hybrid/plant), and each population was arranged in a block, totaling 12 blocks.

Evaluation of production features

Harvesting was conducted on different dates because of different genotype development. Only fruits that were colored dark red over 75% of their area were harvested and measured (Brasil, 2013). The harvested fruits were weighed with an analytical balance and classified as unmarketable (≤ 35 mm) or marketable (>35 mm), according to Brasil (2013).

The end of the marketable production period was fixed when over 70% of the plant under evaluation was producing unmarketable fruits. The total mass (TM) of the fruit (g/plant), the number of marketable fruits (NMF), the number of unmarketable fruits (NUF), and the average fruit mass (AFM) (g/fruit) were then calculated.

Evaluation of physical and chemical characteristics

Firmness was measured from two equidistant points from the center of the fruit using a manual penetrometer with Instrutherm 3mm tip, model PTR-300, and results expressed in Newton (N). The external and internal coloration was determined using the Brasil (2013) scale (1, red-orange; 3, medium red; 5, dark red; 7, blackish red; and 1, red on the edge; 3, red toward the center; 5, uniform red throughout the pulp, respectively).

The soluble solids (SS) level was determined using a benchtop digital refractometer (Reichert AR200) at room temperature and expressed in °Brix. The SS:TA ratio was obtained by dividing the SS readings with the levels (%) of titratable acidity (TA).

Total sugars (TS) were extracted by the Antrona method (Dische, 1962), and determined by spectrophotometry at a wavelength of 620 nm using a standard glucose curve. Total pectin (TP) was extracted with ethyl alcohol (95%) according to a method adapted by McCready and McCoomb (1952), and determined colorimetrically with a carbazole reaction according to the method described by Bitter and Muir (1962).

Statistical analyses

Statistical analyses were performed using Genes software (Cruz, 2013). The heritability of the coefficient of genetic variation and the phenotypic and genotypic covariances (Cruz et al., 2014) were estimated, and the selection gain (%) expected in the selected genotypes in relation to all of the genotypes was obtained by the following expression:

$$GS = \left\{ \left[(X_s - X_o) h^2 \right] 100 \right\} X_o \quad (\text{Equation 1})$$

where GS is the selection gain (%), X_s is the average of the selected genotypes, X_o is the average of all genotypes, and h^2 is the coefficient of genotypic determination (V_g/V_p , where V_g is the effect of genotype and V_p is the total effect of the phenotype). The indices used for the best genotype selection were the classic index (Smith, 1936 and Hazel, 1943), an index based on the sum of ranks (Mulamba and Mock, 1978), and an index based on the distance between each genotype and ideotype (Schwarzbach, 1972 cited by Wricke and Weber, 1986).

The classic index proposed by Smith (1936) and Hazel (1943) consists of a linear combination of multiple characters, in which the weighting coefficients are estimated to maximize their correlation with the aggregated genotype, which is established by another linear combination that involves the genetic values of characters that are weighted by their respective economic weights (Cruz et al., 2014). The classic index is established by the following expression:

$$\mathbf{b} = \mathbf{P} - 1\mathbf{G}\mathbf{a} \quad (\text{Equation 2})$$

where b is the weighting vector index, P^{-1} refers to the inverse of the genetic covariance matrix between characters, G is the genetic covariance matrix, and a is the vector of economic weights assigned to the characters.

The index based on the sum of ranks (Mulamba and Mock, 1978) initially ranks genotypes for each feature by assigning high absolute values to those with better performances. Finally, we added the order of each material for each character, resulting in the following selection index:

$$I = r_1 + r_2 + \dots + r_n \quad (\text{Equation 3})$$

where I is the value for a specific individual, r_j is the rank of an individual in relation to the j^{th} variable, and n is the number of variables considered. The procedure allows the order of the variables to have different weights, as specified by the breeder. Therefore, we have the following expression:

$$I = p_1 r_1 + p_2 r_2 + \dots + p_n r_n \quad (\text{Equation 4})$$

where p_j is the economic weight given by the user to the j^{th} feature.

The genotype-ideotype index is based on the estimation of the distance between the genotypes and an ideotype previously defined by the breeder. Based on this index, the best genotypes are identified and the earnings per selection are calculated. The principal components analysis is performed based on values within a range and the eigenvalues and eigenvectors obtained are associated to the correlation matrix between the analyzed variables Cruz (2006). The calculation of the index is accomplished by the following expression:

$$IDG = \sqrt{\ln \sum Y_{ij} - V O_j^2 n_j} = 1 \quad (\text{Equation 5})$$

Y_{ij} is considered to be the mean transformed phenotypic value, O_j is the optimal value to be resented by the cultivar, under selection. IDG values of Index.

For the genotype-ideotype index, the great values were equal to the average for each character. For the Mulamba and Mock (1978) index, the value of one (1) was adopted for economic weight, in order to check the efficiency of the index. For Smith (1936) and Hazel (1943), different weights were adopted that varied between 1 and 3, according to the importance of the feature to the breeder (Table 2). The selection intensity applied to the three indices was 20% of the hybrids.

Table 2. Characters analyzed and parameters used for the three selection indices.

Variable	Economic weight ¹	Economic weight ²	Great value ³
Total mass (g/plant)	3	1	1023.00
Number of marketable fruits	2	1	43.00
Number of unmarketable fruits	1	1	90.00
Average fruit mass (g/fruit)	1	1	7.00
Soluble solids (°Brix)	1	1	7.00
SS:TA ratio	3	1	7.00
Total sugars (g/100 g)	2	1	6.00
Total pectin (g/100 g)	2	1	388.00
Firmness (N)	2	1	2.00
Internal coloration	1	1	5.00
External coloration	2	1	4.00

¹Smith (1936) and Hazel (1943). ²Mulamba and Mock (1978). ³Genotype-ideotype. SS, soluble solids; TA, titratable acidity.

RESULTS AND DISCUSSION

Table 3 contains the heritability values, and shows that total fruit mass, the NMF, and the NUF had the highest values, particularly the NUF (87.68%).

Table 3. Estimates of heritability (h^2), average of all genotypes (X_o), and average of the selected clones (X_s) obtained by selection indices for the 11 characters analyzed.

Variable	h^2 (%)	Smith (1936) and Hazel (1943)		Genotype-ideotype		Mulamba and Mock (1978)	
		X_s	X_o	X_s	X_o	X_s	X_o
TFM	83.86	1445.83	1023.00	1049.02	1023.00	1101.87	1023.00
NMF	85.86	60.43	43.17	46.79	43.17	53.28	43.17
NUF	87.68	125.12	90.06	80.42	90.06	63.22	90.06
AFM	75.66	7.96	7.94	8.52	7.94	9.86	7.94
SS	60.17	7.34	7.08	7.48	7.08	7.81	7.08
SS:TA	56.23	8.36	7.77	8.69	7.77	9.10	7.77
TS	0	6.00	6.36	8.04	6.36	7.48	6.36
TP	32.22	341.47	388.13	436.04	388.13	434.59	388.13
F	33.96	2.02	2.07	2.16	2.07	2.16	2.07
IC	70.22	5.55	5.47	5.88	5.47	5.95	5.47
EC	70.16	3.98	4.08	4.24	4.08	4.18	4.08

TFM, total fruit mass; NMF, number of marketable fruits; NUF, number of unmarketable fruits; AFM, average fruit mass; SS, soluble solids; TA, titratable acidity; TS, total sugars; TP, total pectin; F, firmness; IC, internal coloration; EC, external coloration.

Heritability values are fundamentally important in breeding programs, because they express the reliability with which the phenotypes represent the genotypes, resulting in an increase in selection reliability (Falconer and Mackay, 1997). The SS and the SS:TA ratio had average heritability values. The combination of these characteristics determines, in part, the flavor of the fruit, which is among the most important attributes in marketing. Taste is a balanced set of different characteristics, and is difficult to measure; however, it is an important feature if you want to select genotypes for their fruit in the *in natura* market (Cantillano, 2006).

Firmness and the TP level had the lowest heritability values, with 33.96 and 32.22%, respectively.

The Smith (1936) and Hazel (1943) indices identified the most important agronomic characteristics with high heritabilities (Table 3), including the TM, NMF, and NUF. The hybrids selected by these indices exhibited increases of 29.3% in TM, 28% in the NMF and a 28% reduction in the number of unmarketable fruits when compared to the original population.

Regarding the physical-chemical characteristics, the genotype-ideotype index selected hybrids that exhibited increases of around 20.9% in TP content and 11% in TS compared to the original population. These characteristics are important in strawberry improvement programs, because they are related to flavor and the fruits' nutritional value (Cantillano, 2006).

For the three indices investigated in this study, the selection frequency was 20% of the hybrids, i.e., of the 103 hybrids evaluated, 20 were selected using selection indices that were based on selection of the characteristics analyzed. Two hybrids were selected by all three methods, showing that regardless of the selection index used, the same hybrids were selected (89 and 495) (Figure 1).

The seven best hybrids selected by the Mulamba and Mock (1978) index were 449, 443, 501, 89, 475, 495, and 214. Hybrid 501 had the highest average NUF (217), AFM (13.24 g), and SS (9.63 °Brix), while hybrid 214 had the highest average TP (692.97 g/100 g).

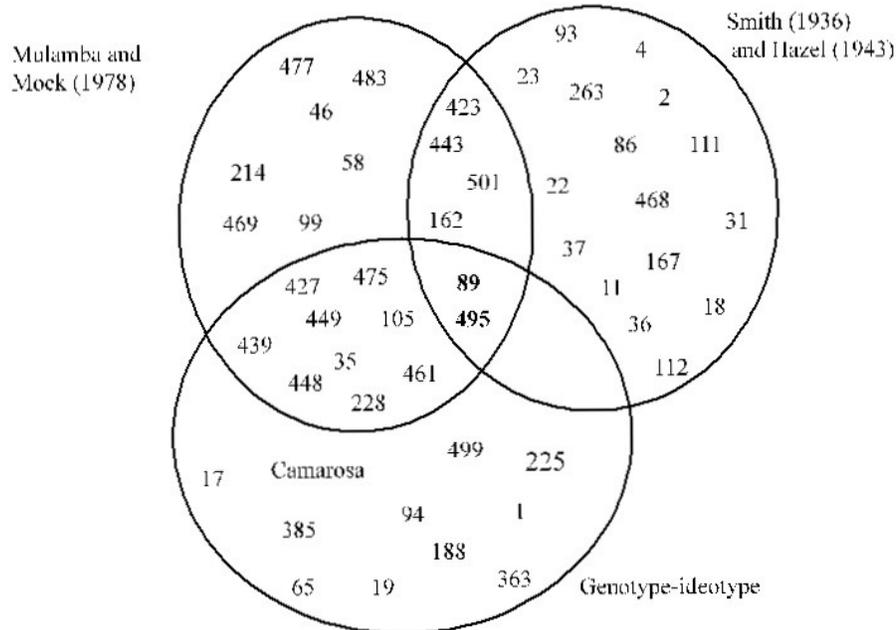


Figure 1. Hybrids selected based on three selection indices: Mulamba and Mock (1978), genotype-ideotype, and Smith (1936) and Hazel (1943).

The Smith (1936) and Hazel (1943) indices selected hybrids with significant gains in TM and NMF, particularly hybrids 93, 443, 37, 4, 11, and 495. Hybrid 443 was also selected by the Mulamba and Mock (1978) index, and had the highest average NMF (103.99). Hybrids 93 and 11 had the highest average TM (2042.47 g/plant) and SS (9.63 °Brix) values, respectively.

The genotype-ideotype index selected a commercial cultivar, ‘Camarosa’ (control), showing the productive potential of the hybrids. Hybrid 225 had the highest average TS (16.07 g/100 g), while hybrid 65 had the highest average firmness (2.98 N). This attribute has great commercial importance, and determines the quality of strawberries and their postharvest use.

The largest genetic gains predicted for most of the characteristics were in hybrids selected by the Mulamba and Mock (1978) index, such as the NMF (20.11%), the AFM (18.24%), the SS (6.16%), and a reduction in the NUF, amongst others. These characteristics are essential from an economic perspective (Table 4).

Table 4. Predicted means (SG%) of hybrids selected based on three selection indices.

Method	Hybrids selected	SG %										
		TM	NMF	NUF	AFM	SS	SS:TA	TS	TP	F	IC	EC
Mulamba and Mock (1978)	449, 443, 501, 89, 475, 448, 427, 495, 105, 58, 46, 162, 461, 228, 35, 439, 477, 99, 423, 483, 214, 469	6.47	20.11	-26.13	18.24	6.16	9.64	0	3.86	1.45	6.15	1.59
Genotype-ideotype	427, Camarosa, 495, 65, 94, 385, 1, 363, 449, 228, 17, 439, 448, 461, 475, 105, 499, 89, 188, 225, 35, 19	2.13	7.19	-9.38	5.49	3.39	6.63	0	3.98	1.41	5.33	2.69
Smith (1936) and Hazel (1943)	93, 443, 37, 4, 11, 36, 495, 89, 22, 167, 468, 31, 86, 111, 111, 112, 263, 23, 501, 162, 423, 18, 2	34.66	34.32	34.14	0.12	2.16	4.26	0	-3.87	-0.87	1.07	-1.85

TM, total mass; NMF, number of marketable fruits; NUF, number of unmarketable fruits; AFM, average fruit mass; SS, soluble solids; TA, titratable acidity; TS, total sugars; TP, total pectin; F, firmness; IC, internal coloration; EC, external coloration.

In a study conducted by Terres et al. (2015) involving three hybrid potato (*S. tuberosum*) populations, estimated total earnings were higher when using the Subandi et al. (1973) and Mulamba and Mock (1978) indices when all of the characters analyzed were considered major. These indices have also been recommended by other authors (Costa et al., 2004; Ribeiro et al., 2012) in obtaining high selection gains.

The Smith (1936) and Hazel (1943) indices showed significant gains in TM and NMF. Using these indices, Amaral Júnior et al. (2010) simultaneously obtained predicted gains for the two main characteristics evaluated (grain yield and capacity expansion) with all economic weights assigned, when studying popcorn (*Zea mays* var. *everta*) populations.

The genotype-ideotype index did not indicate significant gains. TS did not exhibit any selection gain in any of the indices used, possibly because of negative genetic variance.

According to Borém and Miranda (2005), several factors determine genetic variance, such as the characteristics evaluated, the estimation method, the population diversity, the experimental unit considered, and the care with which the experiment is conducted and the data collected. In this study, the negative variance observed could be explained by the quality of the data for this feature, because the design used in the experiment (augmented block) directly influenced the quality of the data.

Selection indices are promising tools for the selection of superior genetic material in plant breeding, because the hybrids selected, particularly those selected by the Mulamba and Mock (1978) index, had several attributes of economic importance. The use of different selection methods allows for greater consistency in selection, particularly when considering the fact that strawberry has an octoploid structure and a high level of heterozygosity, which makes it difficult to separate phenotypic expressions that result from the action of additive components and the dominant and epistatic effects of the environment. Character expression depends upon the genetic control of the organism, the environment in which it is grown, and the interaction between these two factors. Consequently, the probability of success in genetic breeding depends greatly on the inheritance, adaptability, and stability of the characters involved in the program (Antunes et al., 2006); therefore, choosing the right selection index increases breeding program success.

The Mulamba and Mock (1978) and Smith (1936) and Hazel (1943) indices resulted in the greatest gains in the characteristics considered for the strawberry hybrids studied.

The hybrids selected have production potential because most of them had higher values than the commercial cultivars (controls), which will enable the introduction of new strawberry cultivars to the region.

Conflicts of interest

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for their financial support.

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