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Multi-trait selection in ancient grapevine varieties

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Abstract. The selection of ancient grapevine varieties aims to achieve genetic gains in several important traits that can make the variety more interesting for the objectives of the producers. Linear mixed models are fitted to the data to predict the Empirical Best Linear Unbiased Predictors (EBLUP) of genotypic effects for each evaluated trait, which will be the basis for selection. When multi-trait selection is made a method to identify the best genotypes is crucial. The choice of the method is of great importance, since the maximization of genetic gains from selection depends on it. Several important traits were evaluated in field trials of three Portuguese autochthonous varieties. Two different methods, a new selection index and integer programming, were applied for polyclonal selection (a balanced mixture of 7 to 20 clones) and for the selection of individual 30-40 clones to implement the multi-environmental field trials for clonal selection. The results showed that the integer programming method gave better results for polyclonal selection, while the selection index performed better for clonal selection.

1. Introduction

The selection in ancient grapevine varieties seeks to achieve genetic gains in agronomical and oenological traits making these varieties more interesting to the producers. Clonal selection of grapevine varieties began over 100 years ago but has evolved greatly since the 1950s [1]. Since then, clonal selection has led to high genetic erosion of some grapevine varieties [2]. The conservation of genetic intravariability is therefore of paramount importance, as it represents the raw material for future genetic selection. [2, 3]. The Portuguese methodology for the selection of ancient vine varieties [3] was developed with the aim of achieving genetic gains, but also with the concern of preserving intra-varietal genetic variability, through the installation of a large selection field trial with a large number of clones (a representative sample of the variety), which are maintained even after the completion of the selection programme. From this field trial, two types of selection are carried out: polyclonal selection, which consists of selecting groups of 7 to 20 clones that show gains in the most important traits and are grown as balanced mixtures [4], and selection of 30 to 40 individual clones that are used for clonal selection and are selected to be planted in several field trials in different environments to study the interaction between genotype and environment.

Selection should be based on the values of the predictors of genotypic effects rather than phenotypic values [5]. Therefore, to carry out the above-mentioned selections, a large field experiment is conducted with an appropriate experimental design [4]. The empirical best linear unbiased predictor (EBLUP) of the genotypic effect of the different traits is obtained for each clone in each trait by fitting linear mixed models. These values indicate the predicted genotypic effects of a clone for the traits evaluated [4].

Selecting for multiple traits simultaneously, known as multitrait selection, can be difficult and requires a method to identify the top clones. A selection index aims to summarise all the information about a genotype into a single value, allowing all genotypes to be ranked on the basis of their index value, and has been suggested as the most efficient way to perform multitrait selection [6]. However, many of the existing selection indices have some inconveniences, such as the need for economic weight which is difficult to determine [7] or the use of phenotypic values instead of the EBLUP of the genotypic effect. Furthermore, the application of common selection indices can lead to some degradation of some traits [8], particularly if there are correlations between the traits involved that are contrary to the goals of the selection program. In fact, correlations between traits and intra-varietal genetic variability can have a large impact on selection results [2, 9]. In addition, selection indices are designed to select individual genotypes rather than groups of genotypes with the prediction of the overall gains. Based on the idea that different types of selection require different methods, the aim of this work is to compare two selection methods applied to the two types of selection used in a field trial with intra-varietal variability. One of the methods is a new selection index based on the EBLUP of genotypic effect developed in this work, and the other is a method based on integer programming developed for polyclonal selection. These two methods were compared when applied to real data from field trials of three Portuguese grapevine varieties.

2. Materials and Methods

2.1. Plant material and experimental design

To apply the methodologies proposed in this work, data from three grapevine varieties were used (Batoca, a white variety, and Moreto and Tinta Caiada, red varieties). The field trials were planted with a representative sample of the intra-varietal variability of each variety. The experimental design of each field trial, together with the traits and years evaluated for each variety, is described in Table 1.

A sample of 60 berries was collected from each experimental unit the day before harvest. At harvest, yield was determined by weighing the production of each experimental unit. Berry weight, pH, soluble solids content, and total acidity were determined from the berry samples. The anthocyanin content and the total phenolic index were also determined for the red varieties. The results were expressed in kg/plant for yield, g/berry for berry weight, ºBrix for soluble solids content, g Tart. Ac./l for total acidity and mg/g for anthocyanins. To illustrate the application of two multi-trait selection methods, the selection criteria established in this work were: to increase yield, Brix, total acidity, anthocyanins and total phenolics, and to reduce berry weight and pH.

2.2. Linear mixed model

For each trait, the data analysis was based on the average values observed for each experimental unit over the years listed in Table 1. The following model [10] was fitted for each trait of each variety:

$$\boldsymbol{y}_{ijlm} = \mu + \boldsymbol{u}_{gi} + \boldsymbol{u}_{rj} + \boldsymbol{u}_{col(r)jl} + \boldsymbol{u}_{row(r)jm} + \boldsymbol{e}_{ijlm},$$

for $i = 1, \cdots, c,\ j = 1, \cdots, r,\ l = 1, \cdots, s,\ m = 1, \cdots, k$ (*c, r, s,* and *k* for each trait are mentioned in Table 1). The $\boldsymbol{y}_{ijlm}$ are the observations, μ the population mean, $\boldsymbol{u}_{gi}$ the genotypic effects, $\boldsymbol{u}_{rj}$ the resolvable replicate effects, $\boldsymbol{u}_{col(r)jl}$ the column effects within the replicates, $\boldsymbol{u}_{row(r)jm}$ the row effects within the replicates, and $\boldsymbol{e}_{ijlm}$ the random errors. Model effects (except μ) were assumed independent and identically distributed normal variables with zero mean and corresponding variance. Random effects were assumed mutually independent. Linear mixed models were fitted using ASReml-R package [11] for R software [12].

2.3. Quantification of intravarietal variability, prediction of genotypic effects, broad-sense heritability, and predicted genetic gain

The variance components were estimated by the restricted maximum likelihood method (REML) and genotypic variance component was tested using a residual maximum likelihood ratio test (REMLRT) [2]. To compare the quantification of the intra-varietal variability among traits the coefficient of genotypic variation ($\mathrm{CV_G}$) was calculated for each trait. It was computed as the ratio between the estimate of the genotypic standard deviation and the overall mean of the trait [13].

Also important to consider is broad-sense heritability (H^2) [14]. According to Falconer and Mackay [6] heritability is the squared correlation between the predicted and actual genetic effects i.e. a measure of the proportion of phenotypic variation that is explained by genetic causes and thus a measure of the potential success of selection.

Table 1. Experimental design (RCD - resolvable row-column design), traits and years evaluated for each variety.

	Variety		
	Batoca	Moreto	Tinta Caiada
Experimental Design	RCD	RCD	RCD
Number of clones (c)	92	66	220
Number of resolvable replicates (*r*)	5	6	6
Number of rows nested within a resolvable replicate (*k*)	5	11	11
Number of columns nested within a resolvable replicate (*s*)	19	6	16
Plants per experimental unit (*p*)	5	3	3
Traits (years of observation used in the analysis)	Yield (2021, 2022)	Yield, Berry weight, pH (2021 to 2023)	Yield (2020, 2021, 2023)
	Berry weight, pH (2021, 2023)	Brix, Total acidity, Antho-cyanins, Total phenols (2023)	Berry weight, Brix, Total acidity, pH, Antho-cyanins, Total phenols (2020, 2021)
	Brix, Total acidity (2021to 2023)		

Since the EBLUP of the genotypic effect indicates the genetic gain of a clone, the predicted genetic gain of a group of clones is given by the average of the EBLUPs of the genotypic effects of the clones that compose the group [3]. The EBLUP of the genotypic effects of the traits to be reduced (berry weigh and pH) was multiplied by -1 before applying the selection method. Correlation between the EBLUPs of the genotypic effects between each pair of traits was computed for each variety and tested (significance level 0.05).

2.4. Multi-trait selection methods

The selection index proposed in this work (SI) is defined as:

$$SI_j = \prod_{k=1}^{m} \left(\frac{\left| EBLUP_{g_{k_j}} \right|}{\bar{y}_{k..}} + 1 \right)^{r}$$

where SI_j is the index value for clone j, $EBLUP_{g_{k_j}}$ is the EBLUP of the genotypic effect of clone j for the trait *k*, $\bar{y}_{k..}$ is the overall mean of trait *k*, r equal to 1 if $EBLUP_{g_{k_j}} > 0$ and equal to -1 if $EBLUP_{g_{k_j}} < 0$, *m* is the number of traits. The result of this structure is a fraction in which the numerator is composed by the terms with positive and the denominator is composed by the terms with a negative EBLUP of the genotypic effect. Therefore, the greater the positive values of the EBLUPs of the genotypic effects of a clone, the greater the numerator of the index and consequently the index value. The sum of the value 1 in each term prevents denominators with values between 0 and 1.

The Integer Programming (IP) problem used in this [15] work can be written as:

Objective function:

$$Maximize\ z = \sum_{i=1}^{n} \sum_{k=1}^{p_o} y_{k_i} x_i$$

Subject to:

$$\sum_{i=1}^{n} x_i = s$$

$$\sum_{i=1}^{n} y_{k_i} x_i \geq 0\ , for\ \ k = 1, \dots, p_c$$

$$x_i \in \{0,1\}$$

where n is the total number of clones under evaluation; p_o is the number of traits considered for maximization and, therefore, subjected to constraints; y_{k_i} is the value of the EBLUP of genotypic effect divided by the overall mean of the trait k, for clone i; x_i is a binary variable with value 0 if clone i is not selected and 1 if clone i is selected; s is the number of clones to be selected (the size of the polyclonal group); p_c is the number of traits considered for selection constraints.

The two methods were applied to the selection of groups of 7 to 20 clones and groups of 30 to 40 clones. The predicted genetic gains were calculated for each group and compared for groups of the same size in each variety.

A bar chart for the Batoca variety was created using Excel from the EBLUP values of the genotypic effect divided by the overall mean value of the respective trait (stacked bar chart). In this chart, each clone is represented by a bar and each bar contains a fraction related to each trait. The clones appear from top to bottom, sorted by their index value. To better analyze the clones selected by each method, the sets of 7 and 30 clones selected by IP for the Batoca variety are shown in this graph.

3. Results and discussion

Statistically significant intra-varietal variability was found for all of the traits of the different varieties at the 0.05 level of significance (the rejection of the null hypothesis that the genotypic variance component is zero).

Table 2 shows that broad-sense heritability values were between 0.43 in total acidity in the variety Moreto and 0.83 in yield of the variety Tinta Caiada. Heritability is very important because it represents the proportion of phenotypic variability that is due to genetic causes [6]. Therefore, selection cannot be carried out successfully without a good value of heritability.

Table 2. Broad-sense heritability (H^2), overall mean, and coefficient of genotypic variation (CV_G, in percentage of the overall mean).

Variety/Trait	H^2	Overall mean	CV_G (%)
Batoca			
Yield	0.72	1.788	24.54
Berry Weight	0.57	1.87	5.55
Brix	0.52	19.96	2.25
Total acidity	0.46	3.38	4.38
pH	0.45	3.94	0.94
Moreto			
Yield	0.58	6.74	8.14
Berry Weight	0.66	2.51	4.28
Brix	0.70	18.28	6.54
Total acidity	0.43	3.58	6.27
pH	0.63	4.00	0.82
Anthocyanins	0.60	302.43	15.99
Total phenols	0.66	546.56	22.49
Tinta Caiada			
Yield	0.83	4.94	27.45
Berry Weight	0.52	1.79	8.21
Brix	0.78	21.31	5.56
Total acidity	0.44	4.93	5.91
pH	0.70	3.96	1.87
Anthocyanins	0.51	578.46	11.88
Total phenols	0.49	898.41	11.66

The coefficient of genotypic variation is also very important as it allows comparing the estimates of genetic variability between traits of the same variety and the same trait of different varieties [3]. Table 2 shows that the coefficient of genotypic variation was lower in the variety Moreto except for anthocyanins and total phenols and was higher in yield in the varieties Batoca and Tinta Caiada with 24.54 and 27.45, respectively. The lower level of intravarietal variability for the Moreto variety was already mentioned in a previous study [16].

Correlations can be very important for selection results, as high correlations that are contrary to the aims of the selection program can lead to unwanted decreases in some of the traits involved. On the other hand, correlations according to the aim of the selection program can enable indirect selection [6].

Furthermore, null correlations between the traits involved are desirable, as increasing the gain in one trait will not have a negative effect on the other. Table 3 shows the correlations between the traits in the three varieties. For Batoca, Moreto, and Tinta Caiada, 60%, 40%, and 85% of the correlations were statistically significant.

Table 3. Correlations between the several traits in each variety.

			Batoca				
	Yd	Bw	Bx	Ta	pH		
Yd	**1**	0.00	-0.21	**-0.25**	**-0.41**		
Bw		**1**	**-0.37**	**0.26**	**0.32**		
Bx			**1**	**-0.43**	0.17		
Ta				**1**	-0.11		
pH					**1**		
			Moreto				
	Yd	Bw	Bx	Ta	pH	Ac	Tp
Yd	**1**	0.23	0.08	-0.07	**-0.54**	0.15	**0.45**
Bw		**1**	-0.04	0.22	-0.21	-0.14	-0.02
Bx			**1**	**-0.40**	0.07	**0.82**	**0.48**
Ta				**1**	-0.20	**-0.41**	-0.18
pH					**1**	-0.14	**-0.31**
Ac						**1**	**0.68**
Tp							**1**
			Tinta Caiada				
	Yd	Bw	Bx	Ta	pH	Ac	Tp
Yd	**1**	0.03	**-0.57**	-0.12	**-0.53**	**-0.17**	**-0.19**
Bw		**1**	0.11	**0.28**	0.05	**-0.21**	**-0.31**
Bx			**1**	**-0.15**	**0.62**	**0.54**	**0.52**
Ta				**1**	**-0.29**	**-0.25**	**-0.32**
pH					**1**	**0.17**	**0.22**
Ac						**1**	**0.90**
Tp							**1**

Yd - Yield; Bw - Berry weight; Bx - Brix; Ta - Total acidity; Ac - Anthocyanins content; Tp - Total phenolic index. Values in bold represent significant correlations (p-value<0.05)

A high correlation between anthocyanins and total phenols was observed for both red varieties which is expected since anthocyanins are a phenolic compound. Brix also shows high or moderate correlations with anthocyanins and total phenols. For total acidity, most of the correlations are contrary to the objectives. Total acidity is negatively correlated with Brix, which is expected due to the ripening process [9] but goes against the goal of increasing both traits. The negative correlation between total acidity and yield and the positive one with berry weight also contradict the goals of the selection program. The negative correlation between total acidity and pH benefits the objectives and is expected because the higher the acidity the lower the pH. In summary, high values for the coefficient of genotypic variation and heritability increase the possibility of achieving high predicted genetic gains on a trait, especially if that trait has favourable correlations with the other traits.

Polyclonal selection consists of selecting groups of 7 to 20 clones with predicted genetic gains of the group for all traits of interest. Tables 4, 5, and 6 show the results of the predicted genetic gains obtained with the application of the SI and IP in the groups of 7 to 20, 30, and 40 clones for each variety.

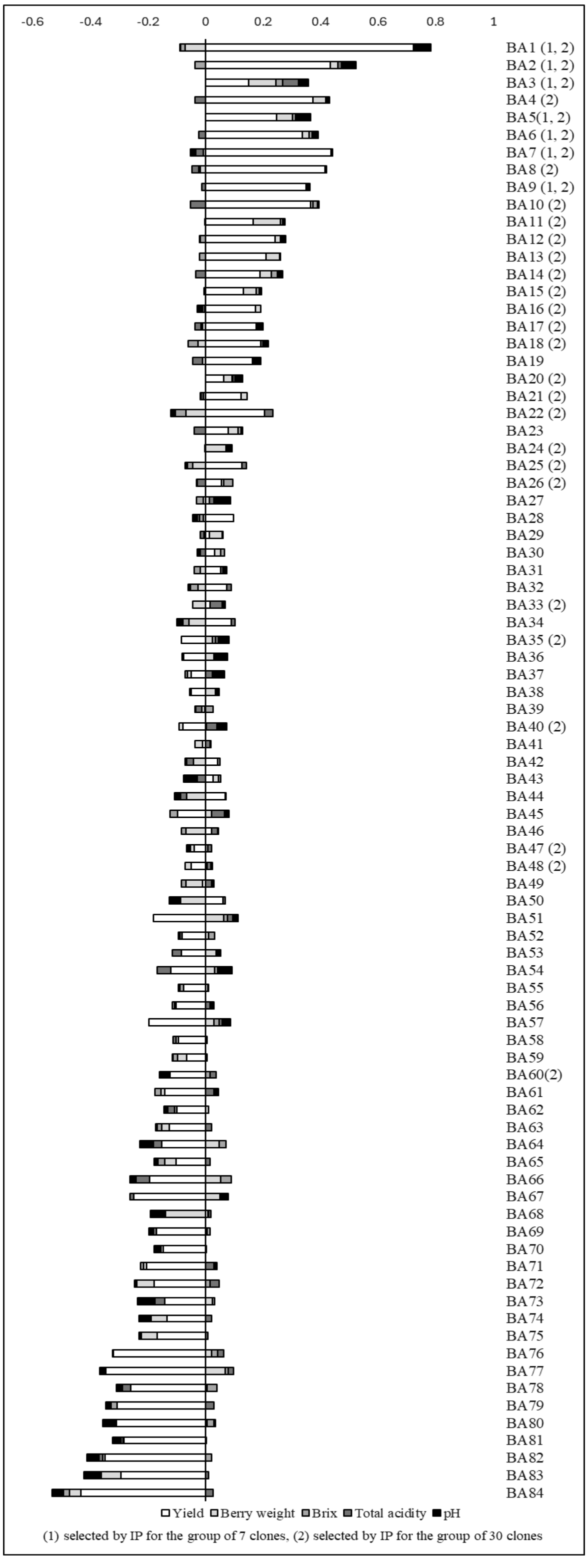
In both cases, higher gains were obtained for yield in the varieties Batoca and Tinta Caiada, and in total phenolic content in the case of variety Moreto which is consistent with the coefficients of genotypic variation obtained. In all varieties, an undesirable reduction in total acidity was observed with SI, which is related not only to the low genotypic coefficients of variation, but also to the correlation between this trait and all the others. The decreases resulting from SI application were also verified in pH and berry weight for the Moreto variety, which is also consistent with the correlations and coefficients of genotypic variation of these traits. Selection indices are developed for selection of individual genotypes (typically to be used in clonal selection) allowing to choose the top clones from a ranking based on the index values. The application of the selection indices can lead to a decrease of some traits and this work shows that this becomes more apparent when the method is applied to the selection of groups of clones. Figure 1 shows the chart obtained for variety Batoca. Each bar refers to a clone. Each fraction of a bar corresponds to a trait and is proportional to the clone's value in that trait. Positive fractions of the bars represent values that are favourable for the goals of the selection program. In this figure, the clones are shown sorted by their index value. The clones at the top of the ranking are those with the larger positive fraction and going down in the charts the positive fraction becomes smaller while the negative fraction becomes larger. Thus, the SI has created a ranking in which the clones with larger positive fractions occupy the top positions, but these clones also have negative portions. In the varieties studied in this work, almost all clones at the top of the ranking of each variety have a negative fraction in total acidity which has led to the decrease observed in the results of the application of the index in this trait.

In contrast to the results obtained with SI, the application of IP was able to achieve gains in all traits in each group for the different varieties. The results obtained with IP allowed no losses in all traits. When IP is applied, the solution consists in the set of clones of the required size that maximize the gains, subject to the constraints that are very important in the case of the problem used in this work to avoid losses since they force gains greater than or equal to zero. Thus, this method manages to overcome, to some extent, the difficulties arising from correlations that are against the objectives, as shown by the results for total acidity in all varieties. However, these difficulties can still be seen in the null gains obtained for this trait.

Table 4. Predicted genetic gains (% of the overall mean) obtained for variety Batoca with the new selection index (SI) and the integer programming (IP).

Obtained with SI					
Number of clones	Yd	Bw	Bx	Ta	pH
7	38.6	2.5	0.1	-0.2	2.9
8	39.0	1.9	0.0	-0.5	2.6
9	38.5	1.8	0.1	-0.6	2.4
10	38.3	1.7	0.2	-1.0	2.2
11	36.4	2.4	0.3	-1.0	2.0
12	35.3	2.3	0.1	-0.9	2.0
13	34.2	2.5	-0.1	-0.8	1.9
14	33.2	2.6	0.1	-1.0	1.9
15	31.8	2.7	0.2	-0.9	1.8
16	30.9	2.7	0.2	-0.9	1.6
17	30.1	2.5	0.1	-1.0	1.6
18	29.5	2.2	-0.1	-0.9	1.6
19	28.9	2.0	-0.1	-1.0	1.7
20	27.7	2.0	-0.1	-0.9	1.7
30	20.9	1.7	-0.2	-0.8	1.2
40	15.4	1.0	-0.3	-0.2	1.3
Obtained with IP					
Number of clones	Yd	Bw	Bx	Ta	pH
7	38.3	1.9	0.0	0.1	0.7
8	36.6	2.3	0.0	0.1	0.6
9	34.0	2.5	0.1	0.0	0.5
10	33.1	1.8	0.1	0.1	0.5
11	31.6	2.2	0.0	0.1	0.5
12	30.7	2.0	0.0	0.0	0.5
13	28.9	2.6	0.1	0.0	0.4
14	28.6	1.9	0.0	0.0	0.4
15	27.7	1.8	0.0	0.0	0.5
16	27.1	1.3	0.1	0.0	0.4
17	25.5	2.4	0.0	0.0	0.4
18	25.2	1.8	0.0	0.0	0.5
19	24.4	1.7	0.0	0.0	0.4
20	23.9	1.7	0.0	0.0	0.4
30	18.5	1.1	0.0	0.0	0.3
40	14.0	0.9	0.0	0.0	0.2
Yd - Yield; Bw - Berry weight; Bx - Brix; Ta - Total acidity.					

Nevertheless, to prevent losses in certain traits, gains in others may be smaller compared to those achieved with SI, as seen in the yield of the Moreto and Batoca varieties However, this is not the case with Tinta Caiada, as higher gains were achieved with IP than with SI in all traits. This finding can be justified by the larger number of clones studied for this variety and the high level of intra-varietal variability observed.



(1) selected by IP for the group of 7 clones, (2) selected by IP for the group of 30 clones

Figure 1. Chart of Batoca clones sorted by SI ranking.

In addition to the ability to prevent losses, the main difference between IP and SI is that with IP, the selected clones are not bound by a predetermined ranking as is the case with SI.

Table 5. Predicted genetic gains (% of the overall mean) obtained for variety Moreto with the new selection index (SI) and the integer programming (IP).

Obtained with SI

Number of clones	Yd	Bw	Bx	Ta	pH	Ac	Tp
7	3.4	-0.5	6.6	-0.7	0.2	16.3	29.6
8	3.0	-0.8	7.0	-0.6	0.2	16.8	27.2
9	2.9	-1.0	6.3	-0.8	0.2	16.5	26.9
10	3.6	-1.0	6.0	-1.9	0.2	15.4	26.4
11	4.2	-1.0	5.1	-1.5	0.3	14.1	25.7
12	3.9	-0.7	4.1	-0.9	0.4	13.0	25.4
13	3.7	-0.3	4.0	-0.9	0.3	12.1	24.8
14	3.5	-0.4	3.9	-0.7	0.4	12.1	23.5
15	3.2	-0.3	3.9	-1.0	0.4	12.5	22.2
16	3.8	-0.4	3.7	-1.2	0.3	11.4	21.9
17	3.4	-0.2	3.9	-1.4	0.3	11.1	21.0
18	2.8	0.0	3.7	-1.3	0.3	11.1	20.1
19	2.4	0.4	3.9	-1.3	0.2	10.7	19.2
20	2.3	0.4	3.9	-1.4	0.2	10.7	18.2
30	2.4	0.2	2.4	-0.9	0.2	7.4	12.7
40	1.3	0.3	1.7	-0.6	0.1	5.0	7.6

Obtained with IP

Number of clones	Yd	Bw	Bx	Ta	pH	Ac	Tp
7	2.5	0.0	4.7	0.5	0.4	14.8	28.8
8	3.1	0.1	3.2	0.1	0.3	13.5	28.5
9	2.2	0.8	4.9	0.0	0.2	13.2	25.8
10	2.6	0.0	5.1	0.0	0.3	13.7	25.7
11	2.7	0.2	4.6	0.4	0.3	12.7	23.7
12	2.7	0.0	4.3	0.1	0.3	12.7	23.8
13	2.5	0.3	4.2	0.0	0.2	11.9	23.3
14	2.4	0.2	4.2	0.1	0.3	11.9	22.1
15	1.7	0.5	4.0	0.0	0.3	11.8	21.0
16	2.4	0.2	3.8	0.0	0.2	10.4	20.7
17	1.6	0.5	3.6	0.0	0.2	11.4	18.8
18	1.9	0.6	3.3	0.0	0.3	10.0	18.8
19	1.9	0.6	3.2	0.0	0.2	9.7	17.8
20	1.2	0.7	3.0	0.0	0.3	10.1	17.0
30	2.1	0.1	1.8	0.0	0.2	6.1	12.6
40	1.5	0.3	1.3	0.0	0.1	4.4	7.5

Yd - Yield; Bw - Berry weight; Bx - Brix; Ta - Total acidity; Ac - Anthocyanins content; Tp - Total phenolic index.

This flexibility in clone selection can be crucial to avoid losses. In fact, in the group of 7 clones of the Batoca and Moreto varieties, only one clone differs between SI and IP but is sufficient to avoid losses of total acidity. When selecting polyclonal material according to the criterion established in this work, it is important to evaluate a group of high-yielding clones, which include clones with high gains in each of the other traits that result in a good overall performance [9].

Table 6. Predicted genetic gains (% of the overall mean) obtained for variety Tinta Caiada with the new selection index (SI) and the integer programming (IP).

Obtained with SI

N. of clones	Yd	Bw	Bx	Ta	pH	Ac	Tp
7	32.4	1.9	0.5	0.6	0.9	7.6	7.9
8	31.0	3.0	0.5	-0.1	0.9	7.8	7.7
9	30.8	3.5	-0.1	-0.6	0.9	7.4	7.9
10	29.8	3.3	0.2	-0.8	0.8	7.6	8.1
11	22.2	2.7	2.8	-0.9	0.6	9.7	11.1
12	23.2	2.6	2.1	-0.4	0.9	9.1	10.2
13	23.0	2.3	2.0	-0.6	0.9	9.3	10.0
14	23.1	2.2	1.8	-0.5	0.9	9.1	9.7
15	24.0	2.2	1.4	-0.7	1.0	8.8	9.2
16	23.8	2.0	1.4	-0.6	1.0	8.8	8.9
17	23.1	1.9	1.5	-0.3	0.9	8.9	8.7
18	22.7	2.0	1.2	-0.2	0.9	8.9	8.6
19	21.6	2.5	1.0	-0.3	0.9	8.9	8.9
20	20.7	2.6	1.1	-0.6	0.9	9.3	8.9
30	19.3	2.6	0.8	-1.0	0.5	8.6	8.6
40	18.6	2.9	0.4	-1.2	0.3	7.8	7.4

Obtained with IP

N. of clones	Yd	Bw	Bx	Ta	pH	Ac	Tp
7	32.4	1.9	0.5	0.6	0.9	7.6	7.9
8	31.0	1.8	0.9	0.2	0.7	7.7	8.1
9	31.4	1.8	0.1	0.7	1.1	7.1	7.2
10	30.4	2.6	0.2	0.2	1.1	7.3	7.1
11	23.4	1.9	2.2	0.0	0.9	9.1	10.6
12	23.5	1.8	2.0	0.0	0.9	8.8	10.2
13	24.5	1.8	1.8	0.0	1.0	8.5	8.9
14	22.2	2.2	2.0	0.0	0.9	9.1	9.4
15	22.4	2.1	1.8	0.0	0.9	8.9	9.2
16	23.8	1.9	1.4	0.1	1.0	8.3	8.5
17	24.5	1.2	1.1	0.0	1.0	8.3	8.2
18	23.6	1.5	1.1	0.1	0.9	8.6	7.9
19	23.6	1.8	0.7	0.0	0.9	8.4	8.0
20	23.9	1.8	0.6	0.0	0.9	8.0	7.6
30	22.7	1.6	0.1	0.0	0.8	7.1	6.4
40	19.6	2.0	0.2	0.0	0.7	6.7	6.3

Yd - Yield; Bw - Berry weight; Bx - Brix; Ta - Total acidity; Ac - Anthocyanins content; Tp - Total phenolic index.

Another major advantage of the IP method is that other values than zero can be chosen for the right-hand side of the IP problem allowing different bounds to be set on the gains of the different traits.

When selecting clones to be used in multi environmental field trials for clonal selection, IP achieved either gains or null results across all traits, while SI produced losses for some traits. To better analyse the clones selected by each method, the groups of 7 and 30 clones selected by the IP for the Batoca variety are shown in Figure 1. Some clones that are not at the top of the ranking are selected to achieve positive gains (or zero) for each trait. In the group of 30 clones, clones BA60 or BA48 are selected based on their contribution to avoid losses in total acidity. However, these clones have a large negative fraction, that is, they are clones with values below the population mean for several traits. On the other hand, with SI, the clones at the top of the ranking are selected, so even the last of the 30 clones (BA30) has a positive fraction greater than the negative one, and no clone has a negative value for yield. Higher genetic intravariability and a higher number of clones involved in the selection program result in a higher number of clones with a larger positive fraction than negative ones. Thus, when selecting individual clones for the multi environmental field trials for clonal selection, SI proved to be a better method than IP, primarily because in this case it is not the group's gains that are important, but the gains of each clone.

In conclusion, the type of selection method used is very important when multiple traits are considered for selection. When applied to polyclonal selection, the IP method performed better than the SI. However, when the two methods were applied to the selection of individual clones to be used in the next stage of selection, better results were obtained with SI. Therefore, in the context of multitrait grapevine selection, integer programming is a method used to select groups of clones maximizing the gain of the group, while selection indices are used to select individual clones.

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