ARTICLE

New approaches using selection index in sweet potato breeding for biofortification

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Hellen Cristina da Silva[1], Valdemir Antonio Peressin[2], José Carlos Feltran[2], Thiago Leandro Factor[2], Eliane Gomes Fabri[2], Luís Carlos Bernacci[2], João Vitor Nomura[1] and Fernando Angelo Piotto[1*]

Abstract: *The objective of this research was to select orange-fleshed sweet potato clones with high stability, yield, and β-carotene content in different planting seasons, using a selection index. Joint analysis of experiments and analysis of responsiveness and stability by the GGE biplot method were performed for the variables yield, dry matter percentage, and root flesh color. The variables yield, dry matter, color and stability were used to estimate a selection index. The GGE biplot analysis indicated that there are differences among clones in stability for Yield. However, the percentage of dry matter and color of the roots show low interaction in the different environments. Because of that, stability for yield was included in the selection index, together with yield, dry matter, and root orange color intensity. Finally, the selection index based on stability, yield, dry matter percentage and root color was efficient in the selection of a new biofortified sweet potato cultivar.*

Keywords: *Ipomoea batatas (L.) Lam., nutritional security, root β-carotene content*

INTRODUCTION

Malnutrition is the unbalanced, deficient or excessive intake of energy as well as nutrients by a person and encompasses two main conditions, overweight and obesity (WHO 2021). During the year 2020 about 2.37 billion people worldwide did not have access to adequate food (FAO et al. 2021) and, in general, zinc, iron, and vitamin A are reported as the principal nutrient deficiencies in global populations. In Brazil the prevalence rates of overweight and obesity exceed malnutrition rates in children, adolescents, and adults, requiring measures that contribute to the consumption of a healthy diet (Canela et al. 2019). Given this, it is possible to state that access to satisfactory amounts of food represents only one factor in ensuring nutritional security, another indispensable aspect is the provision of a diet with adequate nutritional content (Ingram 2020).

Among the approaches for supplying appropriate nutritional levels in a sustainable and cost-effective manner, biofortification stands out (Yadava et al. 2018). Biofortification is the development of food cultivars with high micronutrient contents through conventional breeding, genetic engineering, or through agronomic interventions (Jha and Warkentin 2020). In general, the focus of biofortification is the enrichment with the nutrients iron (Fe), zinc (Zn),

***Corresponding author:**
E-mail: fpiotto@usp.br
ORCID: 0000-0001-9728-6938

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[1] Universidade de São Paulo Escola Superior de Agricultura "Luiz de Queiroz", Avenida Pádua Dias, 11, Agronomia, 13418-900, Piracicaba, SP, Brazil
[2] Instituto Agronômico de Campinas, Avenida Barão de Itapura, 1481, Botafogo, 13075-630, Campinas, SP, Brazil

and β-carotene (Vitamin A). This enables people on low incomes to consume nutritious food on a regular and long-term basis and provides greater access to this food for families in relatively remote rural areas (Nestel et al. 2006).

Among the biofortification strategies, crop breeding is one of the most efficient ways to increase the concentration of some types of nutrients in plants, so that the edible parts are enriched with them and can bring benefits when consumed by humans. Obtaining agricultural crops rich in micronutrients and with good agronomic qualities is technically feasible, and plant species such as maize, wheat, and sweet potato are some successful examples of biofortification (Kiran 2020). In Sub-Saharan Africa, biofortified sweet potato has been widely used for nutritional security, and by the year 2019 one hundred orange-fleshed varieties have been released in 17 countries, which have reached about 6.2 million households (Okello et al. 2019).

The orange-fleshed sweet potato has a large amount of the antioxidant β-carotene, which in the body is converted into vitamin A, a vitamin that aids in the health of the immune system, vision, and skin; the more intense the orange color, the higher the concentration of β-carotene (Low et al. 2017). Sweet potato is a hexaploid species native to Central and South America (Roullier et al. 2013), easily propagated by stems, highly adapted to tropical and subtropical climate conditions, showing great resistance to pests, and could be grown in soils with low fertility, becoming a strategical crop to biofortification approaches. In Brazil, the average yield of the crop is 14.25 tons per hectare (IBGE 2021).

The development of orange-fleshed sweet potato varieties that are adapted to Brazilian regions is an important measure to help reduce malnutrition in the Brazilian population. Thus, this work aimed to select new orange-fleshed sweet potato clones with high stability, high yield, adequate percentage of root dry matter and high β-carotene content, using a selection index.

MATERIAL AND METHODS

The crossing field was installed in the Horticulture Center, of the Instituto Agronômico de Campinas (IAC), in Campinas-SP. Six cultivars were used in the crossbreeding (IAC 2-71 - Americana; IAC 66-118 - Monalisa; SRT 47 - natural variant found within the Beauregard cultivar; SRT 278 - Centenial; SRT 299 - Rio de Janeiro II and SRT 334 - Canadian), with the half-sibling progenies obtained through free pollination. For this, the parents were arranged in predefined arrangements, so that there was the same probability of crossing between them. After three cycles of phenotypic selection, 16 clones with orange flesh were selected and evaluated in different locations and seasons along with three commercial varieties.

The experiments were conducted in the cities of Campinas-SP (lat 22° 52’ 06.0” S, long 47° 04’ 19.1” W), Mococa-SP (lat 21° 26’ 57.3” S, long 46° 59’ 14.3” W), and Piracicaba-SP (lat 22° 42’ 27.9” S, long 47° 38’ 11.0” W). Planting was carried out in three different seasons, in January/February (season A); May (season B) and October/November (season C). Four experiments were conducted in Campinas. The first experiment (Camp-C1) was conducted from November 2018 to March 2019 (average temperature: 23.9 °C, total precipitation: 760 mm); the second (Camp-A1) from February 2019 to August 2019 (average temperature: 20.4 °C, total precipitation: 443 mm); the third (Camp-C2) from October 2019 to March 2020 (average temperature: 23.8 °C, total precipitation: 819 mm) and the fourth (Camp-A2) from January 2020 to June 2020 (average temperature: 20.8 °C, total precipitation: 485 mm). In Mococa, an experiment (Moc-A) was conducted from February 2019 to June 2019 (average temperature: 20.9 °C, total precipitation: 525 mm). In Piracicaba, an experiment (Pir-B) was also conducted with planting in May 2019, which was harvested in January 2020 (average temperature: 22.3 °C, total precipitation: 509 mm). For this work, six experiments were conducted and the effects of developmental seasons and locations on the evaluated traits were studied.

The experimental design used was randomized blocks in all experiments. In the Camp-C1, Moc-A and Pir-B experiments, three repetitions were used with experimental plots of 2.80 m², containing five plants. In the Camp-A1 experiment four repetitions were established with experimental plots of 2.80 m². In Camp-C2 and Camp-A2 five repetitions were established with experimental plots of an area equal to 2.16 m², containing eight plants.

In all experiments, the yield of the clones was evaluated in t ha^{-1} (Yield). Root flesh color was evaluated using two root samples per block (transversal cut in the middle of the root), by means of the colorimetric method of the CIELAB system (L*a*b), measuring in a Konica Minolta colorimeter and roots from the Camp-C1, Camp-A2, Moc-A, and Pir-B experiments. In this work, the variable Root flesh color (COL) was represented by the parameter a* of the L*a*b scale, because this variable is correlated with the concentration of β-carotene in orange-fleshed sweet potato roots (Takahata et al. 1993), and the same has been reported in works with pumpkin (Seroczyńska et al. 2006, Itle and Kabelka 2009). Root dry matter was evaluated in the Camp-A1, Camp-C2 and Moc-A experiments after drying 500g of tuberous roots in a forced air circulation oven at a temperature of 65 °C until a constant mass was reached. Dry matter was calculated using the following formula: DM (%) = $\frac{Dry\ matter}{500} \times 100$.

The joint analysis of experiments was performed to study the genotypes by seasons (GS) interaction and genotypes by locations (GL) interaction. For the joint analysis, a statistical model that best fitted the data was selected based on the lowest value of Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) proposed by Shwarz (1978). The joint analysis for yield and dry matter traits was performed using the following statistical model:

$y_{ijm} = \mu + l_m + g_i + b_{j(m)} + gl_{im} + \varepsilon_{ijm}$, where: y_{ijm} is the observation value referring to genotype *i*, in block *j*, *k* and in location *m*; μ is the overall mean; l_m is the fixed effect of location *m*; g_i is the random effect of genotype *i*; gl_{im} is the random effect of the genotype x location interaction of genotype *i* at location *m*; $b_{j(m)}$ is the random effect of block *j* within location *m*; and ε_{ijm} is the random effect of the experimental error of genotype *i*, in block *j*, and at location *m*, assuming that the errors are independent and normally distributed with zero mean and variance σ^2.

For the flesh color trait, the joint analysis was performed using the following statistical model:

$y_{ikm} = \mu + l_m + g_i + g_{im} + \varepsilon_{ijm}$, where: y_{im} is the observation value referring to genotype *i* at location *m*; μ is the overall mean; l_m is the fixed effect of location *m*; g_i is the random effect of genotype *i*; g_{im} is the random effect of the genotype x location interaction of genotype *i* at location *m*; and g_{im} is the random effect of the experimental error of genotype *i* at location *m*, assuming that the errors are independent and normally distributed with zero mean and variance σ^2.

Variance components were estimated by the restricted maximum likelihood method (REML) (Petterson and Thompson 1971) and genetic values were predicted by the best linear unbiased prediction (BLUP) procedure (Henderson 1975). The predicted means, i.e., BLUP means of the traits yield (Yield), root flesh color (a*), and dry matter (DM) obtained from the joint analysis models were used in adjusting the selection index through the lme4 R package (Bates et al. 2015). For these traits, the Least Significant Difference (LSD, $p < 0.05$) values were presented as a reference for mean comparisons.

The responsiveness and stability analysis of the clones was performed by the GGE Biplot (Genotype and Genotype-Environment Interaction) method in R software, using the GGEBiplotGUI package (Frutos et al. 2014), according to the model:

$G + GE = [(g_i + ge_{ij})]\sum_{s=1}^{n} \lambda_s \gamma_{is} \delta_{js} + \rho_{ij}$, where: G + GE (the mean adjusted for each environment) is the matrix of the effects of genotypes added to the effects of genotype-by-environment interactions; λ_s is the singular value of the *s*-th principal effect; γ_{is} and δ_{js} are the eigenvectors of the *s*-th principal component associated with the effect of genotype *i* and environment *j*, respectively; *n* is the number of principal components retained from the model; and ρ_{ij} is the noise.

Yield stability was determined by evaluating the stability vectors on the GGE Biplot graph, drawing boundaries parallel to the stability line. The clones were then classified into four groups according to their yield stability, and could be considered of very high stability (1 - up to 5 units of deviation from the genotype stability line), high (3 - between 5 and 10 units of deviation from the genotype stability line), intermediate (5 - between 10 and 20 units of deviation from the genotype stability line) or low (7 - above 20 units of deviation from the genotype stability line). This criterion for assigning grades based on stability was established by our research group, to facilitate the use of this criterion in the selection index, prioritizing clones with high stability and penalizing those that showed low stability.

The ordering of the clones was performed using multiple traits through the selection index based on the sum of ranks (Mulamba and Mock 1978). This adaptation was based on the ranking for some variables, by means of groups of means, according to what was described for each variable. Thus, the traits considered in the selection index were yield, root flesh color, dry matter and yield stability.

RESULTS AND DISCUSSION

Table 1 shows the BLUP means for yield, root flesh color and dry matter. It can be seen that the average yield of the clones is higher than the overall mean and the mean of the control varieties, confirming the effectiveness of the selections that have been made in the breeding program. Based on the LSD$_{(p<0.05)}$ value, the clones IAC-465, IAC-484, IAC-641, IAC-691, IAC-725, IAC-737 and IAC-1261 showed superior performance compared to the overall mean and the mean of the control clones.

Regarding root flesh color, the mean of clones was also higher than the overall mean and the mean of the control clones. The clones IAC-86, IAC-484 and IAC-604 showed superior means that did not differ statistically among themselves. Therefore, they are strong candidates for selection as new varieties or as genitors in future crosses, regarding this characteristic. For the dry matter trait, the mean of the clones was close to the overall mean and the difference between the mean of the clones and the mean of the control varieties is relatively low. Interestingly, in sweet potato studies, β-carotene content and dry matter are usually negatively correlated (Mwanga et al. 2017). A possible explanation for this phenomenon is the genetic basis of the negative correlation between β-carotene and starch, in that one of the genes associated with β-carotene content in sweet potato, the phytoene synthase, is physically linked to the sucrose synthase gene, a gene associated with starch biosynthesis (Gemenet et al. 2020). Although there are difficulties in obtaining clones with intense orange flesh and high dry matter content, it is possible to have simultaneous selection gains for these traits in sweet potato (Cervantes-Flores et al. 2011, Mwanga et al. 2017). In Table 1, clones with average dry matter percentages statistically equal to the control means can be observed, such as clones IAC-38, IAC-216, IAC-459, IAC-604, IAC-737 and IAC-1063, which is a good indication that there are clones with satisfactory average dry matter percentages among the genotypes evaluated.

Table 1. Means of yield (**Yield**, in t ha^{-1}), root flesh color (COL, a*) and percentage of dry matter in roots (DM, in %)

Clone	Yield	COL	DM
IAC-38	39.10	18.06	22.43
IAC-86	31.71	23.33	22.31
IAC-216	38.75	17.75	23.73
IAC-459	41.08	12.12	24.75
IAC-465	52.11	18.55	15.94
IAC-484	48.57	22.39	19.11
IAC-579	41.62	16.99	19.08
IAC-596	38.24	19.35	19.69
IAC-604	34.81	22.53	24.15
IAC-641	59.79	19.42	14.76
IAC-691	55.56	18.20	19.02
IAC-698	40.41	17.79	18.59
IAC-725	49.56	19.65	15.12
IAC-737	49.25	15.66	22.57
IAC-1063	41.01	19.68	22.61
IAC-1261	50.48	15.11	18.73
Uruguaiana	36.55	6.63	26.45
Ligeirinha	36.61	-3.85	24.10
Canadense	40.15	-4.47	21.45
Mean	43.44	15.52	20.77
Clone Mean	44.50	18.54	20.16
Control Mean	37.77	-0.56	24.00
LSD$_{(p<0.05)}$ value	13.79	1.98	1.67

As observed in the stability of the clones in relation to the yield trait (Figure 1A, 1B, and 1C), a lot of clones had shown low yield stability, according to other studies with sweet potato production (Gurmu and Mekonen 2018, Karuniawan et al. 2021). The experiments installed in season A showed the lowest overall mean yields of the clones, while the experiments in which planting was performed in seasons C and B showed higher mean yields. The low average yields in season A

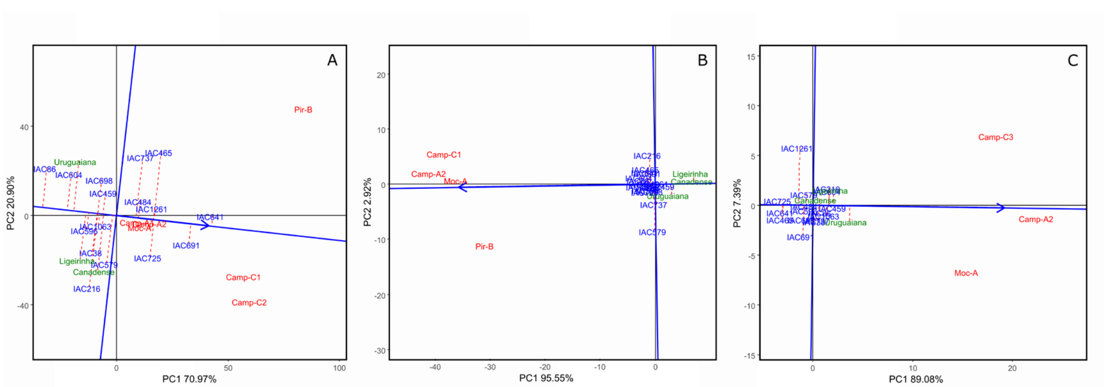

Figure 1. Relationship between mean and stability for A) root yield (t ha^{-1}); B) root flesh color (parameter a*); C) dry matter percentage (%), of orange-fleshed sweet potato clones and controls.

Table 2. Selection index and ranking of biofortified sweet potato clones

Clone	Yield	a*	DM	EST	INDEX	Rank
IAC-1063	10	4	6	1	21	1
IAC-484	7	3	12	3	25	2
IAC-641	1	6	19	1	27	3
IAC-691	2	9	14	3	28	4
IAC-604	18	2	3	5	28	4
IAC-459	9	16	2	3	30	5
IAC-725	5	5	18	5	33	6
IAC-737	6	14	7	7	34	7
IAC-86	19	1	9	5	34	7
IAC-465	3	8	17	7	35	8
IAC-1261	4	15	15	1	35	8
IAC-38	13	10	8	5	36	9
IAC-216	14	12	5	7	38	10
IAC-596	15	7	13	3	38	10
IAC-579	8	13	13	5	39	11
Uruguaiana	17	17	1	5	40	12
IAC-698	11	11	16	5	43	13
Ligeirinha	16	18	4	5	43	13
Canadense	12	19	10	5	46	14

can be explained by the lower temperatures and solar radiation characteristic of this season. Climatic elements such as air temperature, photoperiod and solar radiation are determining factors of growth, development and yield of sweet potato, and too low or too high temperatures can restrict its development. The crop also requires high solar radiation for dry matter formation and root growth (Erpen et al. 2013). Higher stability of the clones was also detected in season A.

Although plants in the experiment of season B faced winter conditions during their initial period of development, the cycle lasted for months in which the environmental conditions were quite advantageous to root yield. The B-season experiment showed the least stability of the clones. The results suggest that seasons B and C were better discriminators of the genotypes for this trait.

In the GGE Biplot referring to the root flesh color it can be observed that the average a* values of the clones are similar, with the exception of the controls, which have lower means than the evaluated clones. Unlike the seasons A and C, in season B the clones were less stable. In general, one of the most important results of this analysis is that the root flesh color characteristic is quite stable, varying little among environments.

The average dry matter values were relatively similar among the clones for the seasons and locations evaluated. Control varieties stood out with higher values of average dry matter, but with low stability. As for the locations, Moc-A promoted lower dry matter values, while in Camp-C3 and Camp-A2 higher means for this trait could be observed. The Uruguaiana cultivar stands out for having a good average percentage of dry matter, but with lower stability compared to the others. The clones IAC-1261 and IAC-691 also stood out from the others for having low stability for dry matter percentage in the environments studied. Besides, they have means below the overall mean.

In Table 2, we observe the final result of the clones' ranking according to the index selection applied. In this ranking, the clone IAC-1063 appears in the first position, where we can see its joint best performance in dry matter percentage, root flesh color and stability. In relation to yield, it was in the tenth position. On the other hand, despite the yield position in the rank, the clone IAC-1063 did not differ statistically from the three control varieties, in its overall mean (Table 1).

In this Index, the most productive genotype, IAC-641, ranked third. Although it has good stability and root flesh color, it was penalized for showing lower dry matter values, being the last clone in this characteristic. As seen previously, dry matter percentage and yield are negatively correlated, and it is difficult to obtain high values for both characteristics in the same clone.

It is also evident that root flesh color, being a trait not correlated with yield and the percentage of dry matter, contributes to gather the best genotypes for this variable in the first positions of the ranking, while yield and percentage of dry matter appeared in contrast in the ranking. Finally, the applied index was efficient to highlight one of the clones that has good yield, a percentage of dry matter within the average of the commercial control varieties, good color and high stability of yield under contrasting environmental conditions. Based on the index, this work indicated the clone IAC-1063 as one of the most promising among the genotypes evaluated.

From the results presented in this research, we conclude that the analysis of responsiveness and stability for yield with orange-fleshed roots showed that there is interaction between clones and environments, and the most significant interactions occurred according to the season of plant development and not in relation to the different locations. The orange flesh root color and the percentage of dry matter in the roots were stable, and their behavior was more predictable in the cultivation of sweet potato clones in different locations and seasons. The selection index was efficient in indicating the clone IAC-1063 as the one that gathers, on average, the characteristics of good yield, percentage of dry matter in roots, orange root flesh, and yield stability. Thus, this clone was registered as a new cultivar with the name "IAC 134 AL01", in honor of the 134th anniversary of the Instituto Agronômico de Campinas (IAC).

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