Proceedings of the 13th ISTRC Symposium, 2007
pp. 746 - 754

Genotypic variation among sweetpotato clones for ß-carotene and sugar content

Mcharo M. and La Bonte D.
Department of Horticulture, Louisiana State University Baton Rouge, LA 70803

Abstract. An F1 population of parents and half-sibs was replicated in field plantings to generate roots to assess levels of ß-carotene, sucrose, maltose, and fructose. DNA was also extracted from each genotype and used to generate AFLP molecular markers. The intent was to identify trait-linked AFLP molecular markers. Genotypes were grouped into phenotypic classes based on their standard deviations from the mean. Association parameters between three classes of each respective sugar and molecular marker profiles were made. Discriminant analysis was found to be a useful tool in building progeny selection models in breeding trials.

Introduction

The importance of the nutritive value of the sweetpotato as human food and animal feed has been well documented by Woolfe (1992). Rubatzky and Yamaguchi (1997) state that as a food, the roots may be roasted, baked, fried or prepared in various combination dishes, providing a rich source of carbohydrates and Pro-vitamin A. Pro-vitamin A or ß-carotene and sugar content have been found to be important consumer traits for various markets (Woolfe, 1992).

Recent studies on root sugar and ß-carotene content have focused on physiological assessment and little on breeding strategies (Bushway, 1986; Picha, 1987; Lu and Sheng, 1990; Katayama *et al*., 1996; K'Osambo *et al.*, 1998 and Labonte *et al*., 2000). According to Picha (1987) carbohydrates constitute most of the dry matter in sweetpotatoes and these carbohydrates exist in the form of starch and sugars. Sucrose, glucose and fructose are the main sugars in raw sweetpotatoes and sweeter baked sweetpotatoes are usually more acceptable to consumers. Picha (1985) also developed reliable HPLC procedures for quantitative analysis of sugars in raw and baked sweetpotatoes. Other methods that have been used for sugar determination include refractive index (Walter, 1992) and near infrared transmittance (Lu and Sheng, 1990; Katayama *et al.*, 1996). Bushway (1986) and Simonne *et al*. (1993) have used HPLC for determination of ß-carotene content. However this methodology is tedious and Takahata *et al*. (1993) have documented a quicker method that links ß-carotene content and the intensity of the orange flesh color of the sweetpotato. ß-carotene has been asociated with prevention of night blindness in children and orange fleshed sweetpotato cultivars being low input crops are therefore attractive sources for ß-carotene for low income societies.

Most of the breeding programmes for cultivars with high ß-carotene have used a classical approach of mass selection. Cultivars that have been developed using this method include 'Jewel' and 'Beauregard'. However, genetic advance in breeding sweetpotatoes with desirable traits is slow and resource consuming.

Hall and Phatak (1993) have reported that the low success in interspecific crosses has precluded the use of genetically related species in sweetpotato improvement. The

hexaploid, self-incompatibility and heterozygous nature of the sweetpotato make inheritance studies very complicated. This genetic condition greatly affects breeding and selection for quantitative traits by slowing down progress in genetic advance within sweetpotato breeding programmes. However the high level of heterozygosity and wide diversity in the germplasm of cultivated sweetpotatoes have also been valuable sources of favorable genes for traits that need to be incorporated through appropriately designed breeding and selection methodologies. Jones (1965, 1986) has proposed breeding methods for many quantitative traits; however, reports on breeding methods that incorporate molecular marker approaches for organoleptic traits are uncommon. Such molecular techniques that would affect interspecific gene transfer could greatly improve quantitative breeding programs (Hall and Phatak, 1993).

Molecular marker assisted selection in sweetpotatoes began with the use of bulked segregant analysis (BSA) (Michelmore *et al.*, 1991) by Ukoskit *et al*. (1997). In their study, Ukoskit *et al*. (1997) identified a primer that could be used to detect linkage between DNA markers and the root-knot nematode resistance gene. The biggest drawback of BSA is the need for new DNA bulks from a homozygous pure F_2 population for every loci targeted. In addition we are not assured of detecting differences between bulks. These hurdles spurred us on to seek alternative approaches to marker assisted selection for sweetpotato improvement. One such approach is the use of a multivariate statistical technique, namely discriminant analysis to associate molecular markers with quantitative traits. Due to the sparsely saturated genome maps in sweetpotato, we seek to develop a method that does not require *a priori* genetic maps and also one that is capable of analyzing multiple traits or variables simultaneously. The multiple variables or molecular markers in our case were generated by AFLP. Discriminant analysis (DA) is a multivariate statistical technique that can identify differences among groups of individuals (or treatments) and improve the understanding of relationships among the variables measured within those groups (Cruz-Castillo *et al*., 1994). DA determines how best to separate or discriminate two or more groups of individuals, given quantitative measurements of several individuals, through simultaneous analysis of several variables (Rencher, 1992). DA finds linear functions of variables that maximally separate two or more groups of individuals while keeping variation within groups as small as possible. DA may be used when it is important to separate known groups or *a priori* groupings, and to identify major sources of difference between groups. Such groups may be determined through a statistical procedure like molecular marker profiling or through cluster analysis techniques. The discriminant analysis will therefore provide information on the discriminatory power of each variable or marker thus determining whether a particular set of variables or markers is useful in separating previously delineated groups (Rojas *et al.*, 2000). We propose to use AFLP and discriminant analyses in a marker-trait association study with the main objective of identifying trait-linked AFLP molecular markers for sugar and ß-carotene content.

Materials and Methods

Planting material. An F_1 population of parents and half-sibs that consisted of 45 clones was used as source material. Maternal parental clones used to obtain the open pollinated F_1 half-sibs were 'Beauregard', 'Excel', '94-96', '86-110', '86-33' and '96-117'. F_1 'Beauregard' progeny were coded as 'BX' while 'Excel' F_1 progeny were coded as 'EL'. The rest of the F_1 progeny were given the codes '96', '110', '33' and '117' to reflect parentage. Field trials were conducted during summer at the Sweetpotato Research Station (Chase), Louisiana in 2001. Each experiment was laid out as a randomized complete block trial with 3 blocks. Each plot consisted of 5 plants with an inter-row spacing of 60cm and

within row spacing of 30cm.Field management followed commercial sweetpotato cultural practices recommended by Boudreaux (1994). The crop was harvested after four months and four to five roots from all the plants in a plot were combined for post-harvest laboratory analysis. The roots were cured at 29^0 C and 90 to 98% relative humidity for about 10 days. Thereafter the roots were stored at 15^0C and 90% relative humidity.

DNA analysis.DNA extraction and DNA analysis using AFLP were done as described by (Mcharo et al, 2003). Young leaves were harvested and stored at -40^0 C until needed. Total DNA was isolated from 100mg of fresh leaf tissue using the Genelute plant genome kit (Sigma-Aldrich Inc., St. Louis, Mo). The DNA samples were amplified in a three step process using a GeneAmp PCR system 9600 thermocycler (Perkin Elmer, Fulerton, CA) using techniques adapted from Vos et al (1995). Four primer pairs were used for selective amplification.

Sugar and ß-carotene analyses. Analyses of sugar and ß-carotene content were done on raw roots stored for 4 to 10 weeks after curing, during which sugar content changes were minimal (Picha, 1987). Sugar content was assayed as described by Picha (1987). Unpeeled roots were halved longitudinally and uniformly grated over the entire surface to a depth of about 3 mm. The grated tissue from each of the 4 roots per replication was combined and 10.0g was homogenized in 80% ethanol for 1 min at high speed using a Brinkman homogenizer (Brinkman Instruments, Westbury, NY). The resulting slurry was immediately boiled for 15 min, cooled, and filtered through Whatman #4 paper. The residue and original container were washed with additional 80% ethanol and the filtrate was made to a final volume of 100ml. Sugar content of the filtrate was determined by high performance liquid chromatography (HPLC) as described by Picha (1985).

Hunter color values (Hunter, 1958) measurements were taken on the flesh of representative cured roots from each plot using a Minolta spectrophotometer cm 3500d (Minolta Co., Osaka, Japan). This color system is based on *L*, *a* and *b* measurements where *L*=lightness, *a*=green-red scale, *b*=blue-yellow scale and intensity. This method measures color by using a positive and negative number scale. For the color value a, a positive value is perceived as red, a negative value as green. For the color value b, a positive value is perceived as yellow, a negative value as blue. Lightness (L) is measured on a scale of 0-100 where 0 = black and 100 = white. Flesh color measurements were taken by measuring the cross-section of the interior of the root.

Statistical analysis. Step discriminant analysis using the STEPDISC procedure (SAS, 2001) was used to select the most informative markers from the original set of markers. The forward selection options in STEPDISC was used to select markers to be included in the classification model. A stop level for the number of markers desired to achieve at least 85% prediction accuracy was imposed to choose the most discriminating markers (SAS, 1999). Wilk's lambda was used as the criterion to determine the classification efficiency with the entry of each marker. The selected markers were then used in a discriminant analysis, DISCRIM option (SAS, 2001), to develop and validate a phenotypic group prediction model and to predict group membership of the test genotypes. The categorical nature of the data could not allow assumption of normal distribution to be made hence a non-parametric method; the k-nearest neighbor method (Rosenblatt, 1956) was used to estimate the group specific densities that produce a classification criterion. The performance of the discriminant criterion was evaluated by group specific error count estimates during cross-validation. This estimator gives the proportion of misclassified observations in each group.

Results and Discussion

Sugar content. Table 1 outlines the sugar content of the various clones studied. Phenotypic grouping of the clones for all sugars was based on the number of standard deviations from the mean. Group 1 contained clones that were equal to or less than negative two standard deviations from the mean. Clones in group 2 were within negative one and positive one standard deviation from the mean while group 3 had consisted of clones at positive two standard deviations or greater. Fructose content ranged from 3.58 ug/20ul of solution in the clone 110-1 to 18.35ug/20ul of solution in BX-8. The same clones had the lowest and highest glucose contents respectively with 110-1 having 6.41ug/20ul and BX-8 containing 30.03 ug/20ul. Clone 110-11 recorded the highest sucrose content at 60.13ug/20ul however the same clone had low scores for fructose (9.31ug/20ul) and glucose (13.31ug/20ul). Labonte et. al. (2000) observed similar sugar content trends in other cultivars. Clone 110-1 had a very high sucrose content while clone BX-8 contained low levels of sucrose. The lowest sucrose content was recorded in clone 117-9 (17.19ug/20ul).

β-carotene content. Investigations by Takahata *et al.* (1993), Ameny and Wilson (1997) and Hagenimana *et al.* (1998) revealed negligible levels of ß-carotene in non-orange cultivars. Takahata *et al*. (1993) studied the relationship between ß-carotene content and Hunter color values in sweetpotato cultivars. They found that color value a* had the highest correlation coefficient (0.891) with ß-carotene and concluded that the color value a* could be used in rapidly estimating the ß-carotene content in breeding programs. The results for the Hunter color value a* are presented in Table 2. The phenotypic grouping was based on the clear demarcation between white, cream-yellow and orange flesh color of clones from visual observation and as represented by the Hunter color value a*. Group1 clones had a white flesh color; group 2 clones had various shades of cream-yellow flesh color while group 3 clones were orange. Clone 96-9 had the lowest a* value (-0.10) and a concomitant high L* value (data not shown). This clone had a white flesh color and was not expected to contain any determinable ß-carotene quantities. Clone 117-7 had a deep orange color and recorded the highest a* value (36.80); however it's L* value was not as high as that of 96-6. A high L* value indicates an increasing lightness in the color hence a lower level of ß-carotene content.

Discriminant analysis. Discriminant analysis (Table 3) elucidated informative markers that could be used for classification of a genotype of unknown grouping. The numerical value for each marker name indicates its molecular weight in base pairs. We noted that markers cag60 and cag203 were important in describing variation for both fructose and glucose. However the same markers were not important in explaining variation for sucrose. Discriminant analysis selected marker cag253 (Table 3) as the most informative in describing variation for ß-carotene content within the samples studied. Table 3 also shows the total number of markers selected to build a predictive model for genotype classification purposes. The objective of selecting the markers was to build models that contained as few markers as possible without compromising on their phenotypic group prediction accuracy. When tested by cross-validation the models achieved a prediction accuracy of at least 85% for fructose and 92% for glucose. ß-carotene needed fewer markers for the discriminant model than the sugars.

Conclusion

Our results indicate the possibility of using molecular markers to predict phenotypic grouping of new genotypes of unknown nutrient composition. We found promising results through the use of the discriminant analysis technique for quick selection during the early stages of sweetpotato breeding. A second year of data is currently being

Table 1: Fructose, glucose and sucrose content and resultant phenotypic classification of sweetpotato clones from an F_1 polycross of 'Beauregard', 'Excel', '94-96', '86-33', '86-33' and '96-117'.

Clone name	Fructose		Glucose		Sucrose	
	mg/g	Group	mg/g	Group	mg/g	Group
110-1	2.0	1	3.0	1	29.5	3
110-11	4.5	2	6.5	2	30.0	3
110-12	3.5	2	5.5	1	16.5	2
110-4	4.5	2	6.5	2	16.0	2
110-5	8.0	3	12.0	2	19.5	2
110-6	2.5	1	4.5	1	21.5	2
110-9	8.0	3	13.5	3	14.5	2
117-1	3.5	2	7.0	2	24.5	2
117-10	5.0	2	10.0	2	16.0	2
117-3	8.5	3	15.5	3	17.5	2
117-5	2.0	1	3.5	1	17.5	2
117-6	4.5	2	10.0	2	16.0	2
117-7	4.0	2	7.5	2	19.5	2
117-8	4.5	2	9.5	2	17.0	2
117-9	8.5	3	14.5	3	8.5	1
117p	4.5	2	7.5	2	22.0	2
33-1	7.0	2	12.0	2	18.0	2
33-10	4.0	2	6.5	2	27.5	3
33-11	3.0	1	5.0	1	23.5	2
33-3	9.5	3	15.0	3	20.0	2
33-7	6.0	2	10.5	2	16.0	2
33-8	8.5	3	14.6	3	23.0	2
33-9	8.5	3	13.5	3	17.5	2
96-10	5.0	2	11.5	2	29.5	3
96-11	5.5	2	7.5	2	30.0	3
96-2	3.5	2	6.5	2	23.5	2
96-4	5.5	2	10.5	2	25.5	3
96-5	4.0	2	7.5	2	22.0	2
96-7	7.0	2	12.5	2	23.5	2
96-8	2.0	1	3.5	1	27.5	3
96-9	5.5	2	10.0	2	11.0	1
96p	7.0	2	11.0	2	23.0	2
BX-1	8.5	3	13.5	3	10.0	1
BX-16	7.0	2	12.0	2	21.0	2
BX-2	7.0	2	11.0	2	16.5	2
BX-21	3.0	1	5.0	1	15.5	2
BX-5	7.0	2	11.0	2	22.5	2
BX-6	6.5	2	11.5	2	15.0	2
BX-8	9.0	3	15.0	3	12.5	1
BXp	7.0	2	12.0	2	11.5	1
EL-11	6.5	2	15.5	3	16.0	2
EL-4	2.5	1	4.5	1	14.5	3
EL-5	7.5	2	15.0	3	9.5	1
EL-7	9.0	3	13.5	3	16.0	2
EL-8	3.5	1	6.0	1	20.5	2

Clones followed by the letter 'p' are parental clones.

Table 2: Hunter color value a* and phenotypic classification for ß–carotene content of sweetoptato clones from an F_1 polycross of 'Beauregard', 'Excel', '94-96', '86-33', '86-33' and '96-117'.

Clone name	Color value a*	Phenotypic group
110-1	29.47	3
110-11	31.79	3
110-12	15.63	2
110-4	30.92	3
110-5	31.22	3
110-6	30.46	3
110-9	24.45	3
117p	36.51	3
117-1	33.47	3
117-10	32.21	3
117-3	30.50	3
117-5	33.77	3
117-6	28.73	3
117-7	36.80	3
117-8	18.96	2
117-9	2.13	1
33-1	21.57	3
33-10	3.19	1
33-3	33.48	3
33-7	28.01	3
33-8	25.84	3
96-10	2.27	1
96-11	30.25	3
96-2	9.67	1
96-4	22.83	3
96-7	17.45	2
96-8	31.46	3
96-9	-0.10	1
96p	31.40	3
BX-1	5.00	1
BX-16	13.43	2
BX-2	24.20	3
BX-5	31.18	3
BX-8	33.96	3
BXp	27.99	3
EL-11	11.92	1
EL-4	33.09	3
EL-5	0.89	1
EL-7	30.63	3
EL-8	14.90	2

Clones followed by the letter 'p' are parental clones.

Table 3: Four most informative markers for sugar content and ß–carotene in sweetpotato.

Trait		Total markers selected	4 most important markers selected	Wilk's Lambda	Pr < Lambda
Sugars	Fructose	14	cag203	0.72	0.0023
			cag60	0.50	<0.0001
			cag95	0.35	<0.0001
			cag147	0.28	<0.0001
	Glucose	13	cag240	0.74	0.004
			cag203	0.57	0.0004
			cag60	0.43	<0.0001
			cag192	0.33	<0.0001
	Sucrose	11	cag206	0.71	0.0018
			cag122	0.52	<0.0001
			cag91	0.37	<0.0001
			cag119	0.27	<0.0001
ß–carotene		10	cag253	0.68	0.0007
			cag91	0.52	<0.0001
			cag94	0.40	<0.0001
			cag242	0.31	<0.0001

Table 4: Error count estimates from cross-validation using nearest neighbor for various traits in sweetpotato.

	Phenotypic group			Total
	1	2	3	
Fructose	0.33	0.12	0.00	0.15
Glucose	0.14	0.09	0.00	0.08
Sucrose	0.17	0.00	0.13	0.10
ß–carotene	0.12	0.20	0.04	0.12

analyzed to more fully document our preliminary findings.

Acknowledgments

The authors express appreciation to The McKnight Foundation, Minneapolis, Minnesota, USA and state and matching funds allocated to the Louisiana State University Agricultural Center for support of this research.

References

Ameny, M.A., and P.W. Wilson. 1997. Relationship between Hunter color and ß – carotene content in white-fleshed African sweetpotato. J. Sci. Food Agric. 73:301-306.

Boudreaux, J.E. 1994. Commercial vegetable production recommendations. La. Coop. Ext. Serv. Publ. 2433.

Bushway, R. J. 1986. Determination of á and ß - carotenoids in fruits and vegetables by

high performance liquid chromatography. J. Agri. Food Chem. 34:409 412.

Cruz-Castillo, J.G., Ganeshanandam, S., MacKay, B.R., Lawes, G.S., Lawoko, C.R.O. and Woolley, D.J. 1994. Applications of canonical discriminant analysis in horticultural research. Hortscience 29:1115-1119.

Hagenimana, V., L.M. K'Osambo, and E.E. Carey. 1998. Potential of Sweetpotato in reducing vitamin A deficiency in Africa. CIP Program Report. pp287-294.

Hall, M. R and S.C Phatak. 1993. Sweetpotato [*Ipomoea batatas* (L.) Lam.], p 693-708 In: *Genetic Improvement of vegetable crops*. Kalloo G and B.O Bergh (eEds). Pergamon Press.

Hunter, P.S. 1958. Photoelectric color difference meter. Journal Optical Society of America 48:485-995.

Jones, A. 1965. Cytological observations and fertility measurements of sweetpotato [*Ipomoea batatas* (L.) Lam.], Proc. Am. Soc. Hortic. Sci. 86:527-537.

Jones, A. 1986. Sweetpotato heritability estimates and their use in breeding. Hortscience 21:14-17

Katayama, K., K. Komaki, and S. Tamiya. 1996. Prediction of starch, moisture, and sugar in sweetpotato by near infrared transmittance. Hortscience 31(6):1003-1006.

K'osambo, L.M., E.E. Carey, A.K. Misra, J. Wilkes, and V. Hagenimana. 1998. Influence of age, farming site and boiling on pro vitamin A content in sweetpotato [*Ipomoea batatas* (L.) Lam.] storage roots. J. Food Composition and Analysis 11: 305 321.

Labonte, D.R., D.H. Picha, and H.A. Johnson. 2000. Carbohydrate-related changes in sweetpotato storage roots during development. J. Amer. Soc. Hort. Sci. 125(2):200-204.

Lu, G.Q. and J.L. Sheng. 1990. Application of near infrared reflectance spectroscopy (NIRS) in sweetpotato quality breeding. Scientia Agricultura Sinica 23(1):76-81.

Mcharo, M., E. Bush, D. La Bonte, C. Broussard, and L. Urbatsch. 2003. Molecular and Morphological Investigation of Ornamental Liriopogons . Amer. Soc. Hort. Sci. Vol. 128(4): 575-577.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulk segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9829-9832.

Picha, D.H. 1985. HPLC determination of sugars in raw and baked sweetpotatoes. J. Food Sci. 50:1189-1190.

Picha, D.H. 1987. Carbohydrate changes in sweetpotatoes during curing and storage. J. Amer. Soc. Hort. Sci. 112:89 92.

Rencher, A.C. 1992. Interpretation of canonical discriminant functions, canonical variates, and principal components. Amer. Statistician 46:217-225.

Rojas, W., Barriga, P. and H. Figueroa, H. 2000. Multivariate analysis of Bolivian quinua germplasm. Plant Genetic Resources Newsletter. No 122:16-23.

Rosenblatt, M. 1956. Remarks on some nonparametric estimates of a density function. Annals of Mathematical Statistics. 27:832-837

Rubatzky, V.E and M. Yamaguchi, M. 1997. Sweetpotato, p 130-146. In: World vegetables, principles, production and nutritive values. AVI. Van Nostrand Reinhold Company, New York.

SAS Institute Inc. 1999. SAS/STAT User's guide. SAS Institute Inc. Cary, North Carolina.

SAS Institute Inc. 2001. The SAS system for windows v8. SAS Institute Inc. Cary, North Carolina.

Simonne, A.H., S.J. Kays, P.E. Koehler and R.R. Eitenmiller. 1993. Assessment of ß-carotene content in sweetpotato breeding lines in relation to dietary requirements. Journal of food composition and analysis 6:336-345.

Takahata, Y., T. Noda, and T. Nagata. 1993. HPLC determination of ß – carotene of sweetpotato cultivars and its relationship with color values. Japanese J. Breed 43: 421-427.

Ukoskit, K., P.G. Thompson, C.E. Watson Jr., and G.W. Lawrence. 1997. Identifying a randomly amplified polymorphic DNA (RAPD) marker linked to a gene for root-knot nematode resistance in sweetpotato. J. Amer. Soc. Hort. Sci. 122:818-821.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Walter, W.M.1992. Use of refractive index to monitor changes in sugar content of stored sweetpotatoes. HortScience 27(4):333-335.